Vol. LIV. *April, 1928.* *No. 4.*

BIOLOGICAL BULLETIN

GRAFTING AND REINCORPORATION IN ACTINOSPHÆRIUM EICHHORNII EHR.

RUTH B. HOWLAND,

WASHINGTON SQUARE COLLEGE, NEW YORK CITY.

It is a well known fact that contiguous individuals [1] in a culture of *Actinosphærium eichhornii* may fuse along the surfaces in contact. Cytoplasmic union of this type, or plastogamy,[2] has been frequently described, and has often been considered as an important initial step in the evolution of nuclear union.

In the closely allied form *Actinophrys sol*, plastogamy is a preliminary step toward nuclear fusion. A striking photomicrograph of this process, taken from living material, is shown by Bělař, '22, (Taf. 3, Fig. 24). Fusion of a number of these individuals around a large food mass, has recently been described by Looper, '25. This union is temporary, for separation occurs as soon as digestion is completed.

Attempts to produce plastogamous fusion artificially have met with varying degrees of success. Cienkowski, '65, was able to fuse two vegetative masses of *Actinosphærium* if he brought together two cut surfaces, and held them together with paper strips. Johnson, '94, could not bring about artificial coalescence in a single instance. Greff, '67, and Penard, '04, observed that a single vegetative mass, if fragmented into a large number of portions under a cover slip, might eventually fuse into the original form. In some cases, this may have been a simple contraction phenomenon, for no attempt was made to break apart the cytoplasmic threads connecting the crushed fragments.

[1] In behavior, the single sphere commonly termed an individual *Actinosphærium*, shows all the characteristics of a colonial aggregate. This would seem the more accurate terminology to apply to each vegetative mass.

[2] Also spelled plasmogamy. Bělař, '22.

In the course of an extended physiological study of Actinosphærium eichhornii, it was observed that artificial plastogamy could invariably be induced by suspending two or more animals in a hanging drop small enough to exert moderate compression. Various types of simple grafts were found to be successful when sufficient pressure was exerted by this method to enforce temporary contact.

Also, the interesting behavior of axopods[3] which were occasionally severed from the main body during the transfer of animals to hanging drops, seemed worthy of critical attention. Reincorporation of pseudopodial fragments has been reported in a few instances. Jensen, '96, and '01, describes this phenomenon in *Orbitolites* and *Amphistegina*. Kepner and Reynolds, '23, observed in *Difflugia* the recovery of separated pseudopodial fragments by their cell bodies.

The results derived from these accidental and experimental graftings, and from the severing of axopods, in *Actinosphaærium* are presented in the present paper.

Material and Methods.

Actinosphærium eichhornii was reared in large numbers in a medium containing ciliates, rotifers and round worms. Subcultures were made in Great Bear Spring Water, to which had been added concentrations of *Colpoda, Paramecium, Noteus* and *Philodina*.

Gross merotomy was performed under a binocular microscope. To aid in differentiating between the cut portions of two individuals, half of the animals to be grafted were stained in a very dilute solution of neutral red, made up in the original medium. The neutral red penetrated easily, and stained the cytoplasmic granules a deep red. A stained (red) and an unstained (white) animal were placed in a Syracuse watch glass, and cut into halves or quarters with a glass hair. Before normal contraction could cause rounding of these portions, two or more were transferred to

[3] Greeff (*loc. cit.*) saw "unipolar, pear-shaped cells," among the fragments resulting from his crushing experiments. In all probability, these were derived from axopodia, but their origin could not be accurately observed when so drastic a method of separation was applied.

a hanging drop, the size of which was reduced until the animals came into contact at the desired angle. These were transferred to a moist chamber for observation under high power (Leitz oc. 8x, obj. 7a).

Severing of individual axopodia was accomplished by means of the Chambers micrurgical apparatus, and the subsequent behavior followed under high power.

Results.

(*a*) *Grafting of Cut Portions.*

Grafted animals fused perfectly in all cases. The portions taken from each of the two contributing animals remained distinctly marked off. The surfaces held in contact varied in position and extent.

Record 3, (Text-figures 1 to 7), covers briefly the range of possible variations. In Figs. 1 and 2, two cut medullary surfaces (*M–M*) were brought into contact. Two quadrants of a stained individual brought into contact, medulla to medulla, (*M–M*), and medulla to cortex, (*m–C*), are shown in Figs. 3, 4 and 5. Figs. 6 and 7 represent a case in which an entire stained (red) animal was grafted on to half of an unstained (white) individual.

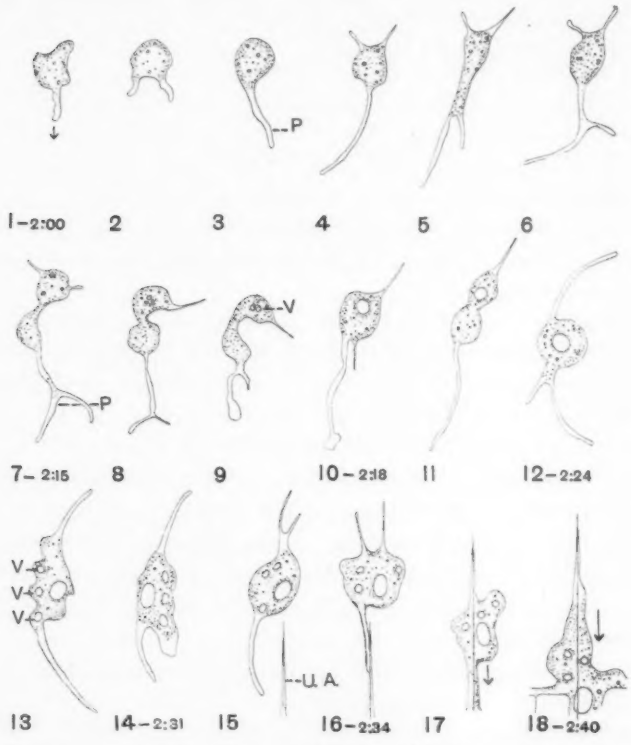
The time taken for complete fusion of surfaces varied from five to twenty minutes. As the animals were returned to the deeper water in a Syracuse watch glass, they resumed their normal spherical shape.

The extent and position of the grafted portions was still recognizable at the end of thirty hours.

(*b*) *Merotomy and Reincorporation of Axopodia.*

Axopodia were severed from the main body, carried away by the needle for some distance to break all connecting strands of protoplasm, and returned to the immediate region of the animal. Freed from the microneedle, they exhibited slow, erratic, swinging movements. If these movements brought them into chance contact with an unsevered axopod, or with the cortical surface of the main body, they were incorporated (Records 1, 2 and 5).

The first step after severance is usually the rounding up of the broad basal end of the axopod as an irregular granular mass, into which the extended portion is gradually withdrawn (Record 4; Figs. 1, 2, 3), and from which new pseudopodial extensions of

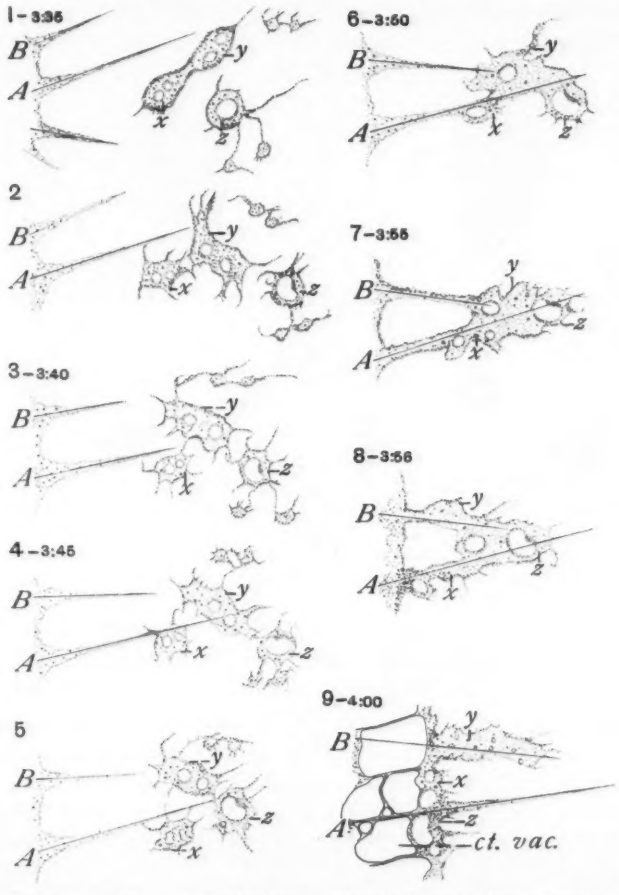


RECORD 1, Figs. 1–18. Changes of form exhibited by a severed axopod before reincorporation. Time lapse, 2:00–2:40. *P*, pseudopodial extensions. *V*, newly formed vacuoles. *U. A.*, axopod still unsevered from the main body.

great length are thrown out (Fig. 4). During its migration, this body becomes increasingly vacuolated (Record 1, Figs. 10–18). No axial cores were ever observed in its pseudopodia (filopodia).

A number of severed axopodia may unite to form a loose network (Record 2, Figs. 1 to 9), which behaves as a single unit.

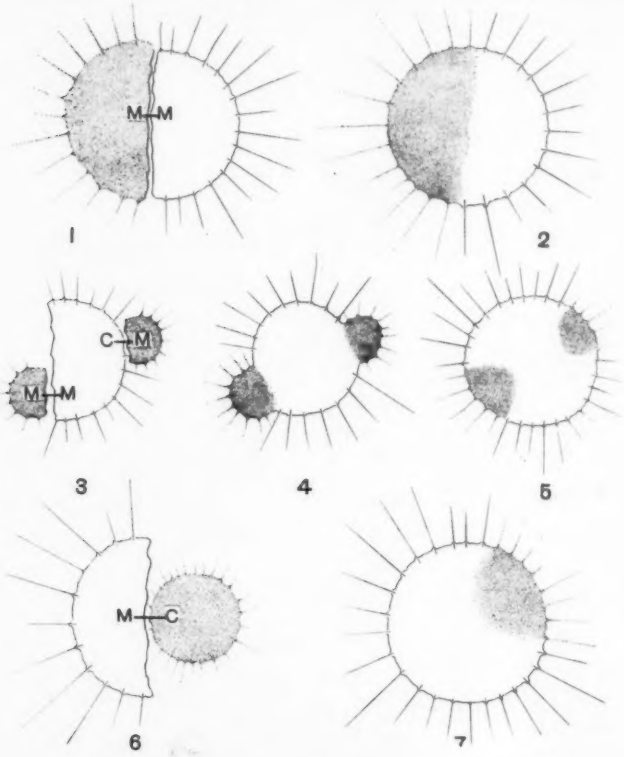
The first sign of protoplasmic union may occur at either the tip or the side of an axopod. Fragments reincorporated near the



RECORD 2. Figs. 1–9. Protoplasmic network formed from a number of severed axopodia. *x*, *y*, *z*, individual axopodia, gradually fusing, and finally reincorporated as a single mass. Time lapse, 3:35 to 4:00. *A* and *B*, the two unsevered axopodia chiefly concerned in the reincorporation process. *Ct. vac.*, vacuoles of the cortical layer of the main body.

tip, increase the size of an axopod at that point to several times the original diameter, and have the appearance of a large lateral

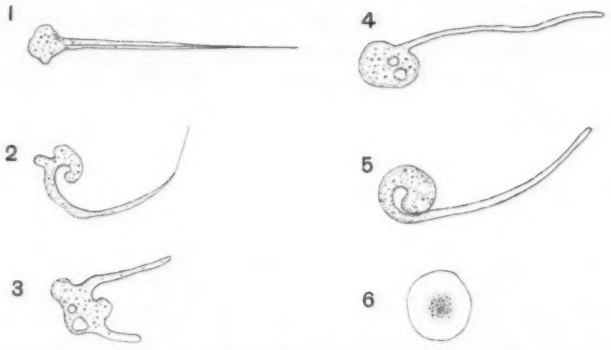
swelling (Record 5, Fig. 6). This mass travels centripetally, flattening as it goes, and fuses completely at the level of the cortical surface (Record 5, Fig. 7; Record 1, Fig. 18).



RECORD 3, Figs. 1–7. Diagrammatic representations of different types of grafting between stained and unstained vegetative masses of *Actinosphærium eichhornii*. Stippled areas represent portions of animals stained with dilute neutral red. Clear areas represent portions of unstained animals (white). *M–M*, medulla grafted to medulla. *C–M*, cortex grafted to medulla.

Axopodia cut from one animal and carried on the needle point to the immediate region of another, will be incorporated if the protoplasm is still capable of locomotion. Dead (motionless) axopodial masses are refused.

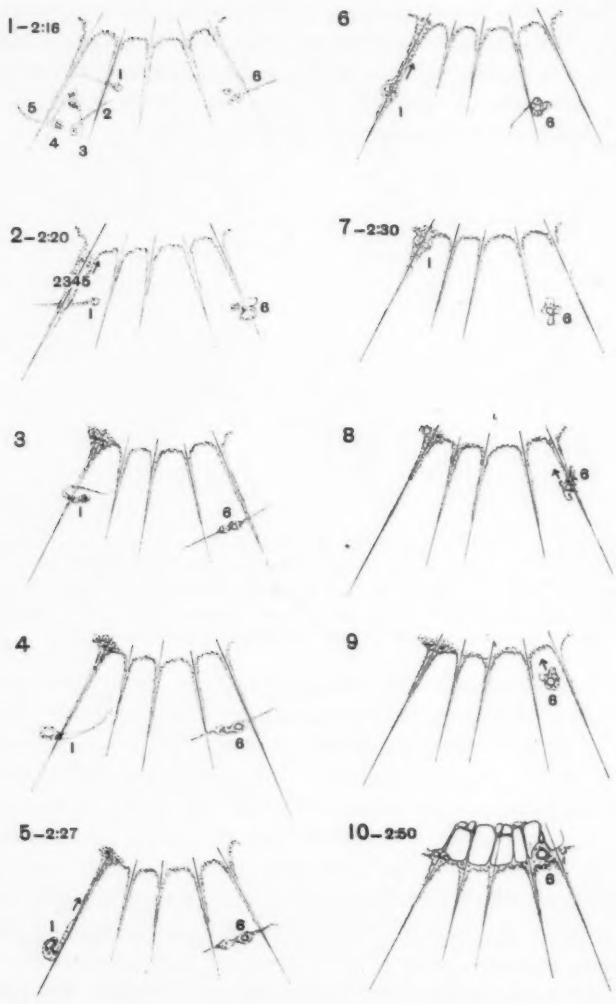
Frequently, severed axopodia were observed which approached very closely to an animal without fusing, and subsequently moved away. If fusion had not occurred at the end of 40 to 50 minutes, the pieces became spherical and quiescent, and showed signs of hyalinization and disintegration (Record 4, Fig. 6).



RECORD 4. Figure 1 represents an axopod just severed from the main body. Figs. 2 and 3, stages during resorption of the original axopodial extension. Fig. 4, newly formed pseudopod, without axial core. Fig. 5, twisting movement which results in a clumsy locomotion. Fig. 6, hyalinization and disintegration after failure to become reincorporated.

It is demonstrated in the foregoing experiments that the highly vacuolated cytoplasm of the vegetative masses (colonial aggregates) of *Actinosphærium* is very adhesive. The ease with which artificial plastogamy or grafting is accomplished under compression, strongly suggests the possibility that the failure of Johnson, '94, to fuse vegetative individuals was due to the lack of some similar device. Also, the essential factor in the experiments of Cienkowski, '65, would seem to be the compression which he applied by the use of paper strips, rather than the approximation of two cut surfaces.

Reincorporation of severed axopodia is, in reality, the same process on an infinitely smaller scale. The fusion, in these cases, is unlike reincorporation in *Difflugia* in that it may occur at the tip or side of an unsevered axopod, or at the cortical surface of the main body.



RECORD 5. Figs. 1–10. Diagrammatic representation of the process of reincorporation of six severed axopodia, numerically labelled. Time lapse for reincorporation of numbers 2, 3, 4 and 5, was four minutes; for number 1, fourteen minutes. Number 6 did not fuse with unsevered axopodia, but moved slowly toward the cortex, fusing there in thirty-four minutes.

SUMMARY.

Grafting.

1. Merotomized portions of *Actinosphærium eichhornii* may be grafted together under slight compression.

2. Fusion occurs between two medullary, or between medullary and cortical surfaces.

3. Grafted animals regain a spherical shape, and show no sign of separation at the end of four days.

Reincorporation of Axopodia.

1. Axopodia completely severed from the protoplasmic mass may be reincorporated, either by the same or by another animal.

2. Severed axopodia often become vacuolated, and develop long pseudopodia.

3. Reincorporation may occur at either the tip or the sides of unsevered axopodia, or on the cortical surface of the main body.

4. Severed axopodia which have not been reincorporated at the end of 40 to 50 minutes, round up, become quiescent, and show signs of disintegration.

5. Motionless axopodial fragments are not incorporated.

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MOSAICISM AND MUTATION IN *HABROBRACON*.

P. W. WHITING,

BUSSEY INSTITUTION, HARVARD UNIVERSITY.[1]

In a recent publication (Whiting and Whiting, 1927) the hypothesis was advanced that certain irregular types in the parasitic wasp, *Habrobracon*, were caused by incomplete maturation of the egg. Impaternate females were assumed to originate from eggs in which there was failure of second maturation division. Mosaic males from unfertilized eggs of heterozygous mothers were thought to be due to failure of extrusion of second polar body, two oötids with different genes taking part in maturation; gynandromorphs to have originated in a manner similar to that of the mosaic males, except that one oötid was fertilized. A single heterozygous mosaic female was explained by mitotic irregularity in somatogenesis.

In the present paper are presented data giving additional evidence for the hypotheses previously advanced although no more impaternate females have been found. Factors considered here are the allelomorphs black, O, light, o^l, orange, o, and ivory, o^i (eye color), (Whiting and Burton, 1926) and normal, R, and reduced, r, (wings), (Whiting, 1926). Materials involved are stock 11, (type) from Iowa City, Iowa, which when previously used in crosses with Lancaster material gave the irregular types already discussed; stock 17 (ivory) and stock 20 (ivory reduced), both of mixed origin from the Lancaster and Iowa City materials; stock 24 (type) from Lowell, Massachusetts; and orange-eyed males from various stocks.

Stock 24 has not been previously described. A female, captured September 7, 1926 in Lowell, produced 24 males and 73 females. Thirteen virgin females produced 651 males. Thirty-

[1] The present work, begun at the University of Maine, has been carried on at the Bussey Institute under a grant from the Committee for Investigation of Problems of Sex of the National Research Council. The writer wishes to express his indebtedness to this committee for support and to the Bussey Institution for courtesy in extending space and facilities.

TABLE I.

PROGENY FROM Oo^1Rr FEMALES, VIRGIN OR CROSSED WITH VARIOUS MALES.

Parents.			Regular Offspring.								Freaks.		
F_1 ♀ ♀ Oo^1Rr.		♂♂.	♂♂.				♀♀.				Biparental ♂♂.	Mosaic and Mutant ♂♂.	♀♂.
No.	Source ♀ ♂		Type.	Ivory.	Reduced.	Ivory Reduced.	Type.	Orange.	Reduced.	Orange Reduced.			
24	20 × 11		994	989	940	934						283, 289, 319	
14	11 × 20		468	494	417	449						286, 287	
9	11 × 20	Orange	64	73	62	54	322	296			292	305	303
7	11 × 20	Orange Reduced	81	76	60	60	141	98	114	128	290, 291, 294		296, 302
6 6	20 × 24 24 × 20		308	315	277	298							
25 12	20 × 24 24 × 20	Orange	430	465	414	415	1,207	1,201			293?, 297, 298	306	
3 6	20 × 24 24 × 20	Orange Reduced	126	123	107	98	96	104	55	70	293a?, 295		
3	20 × 11	Ivory Reduced	55	52	36	46	100	Ivory 109	94	Ivory 89			
Total regular showing segregation.....			2,526	2,587	2,313	2,354	337	311	263	287			

six mated females produced 1417 males and 1129 females. These wasps were of normal appearance, like Lancaster "type" stock 1, but mesosternum was very "sooty" and 9 males and 6 females had breaks in wing vein r_4.

Reciprocal crosses were made between stocks 20 and 11 and between stocks 20 and 24. The F_1 females were in some cases isolated as virgin, in others bred to orange males (stocks 3, 12, 13) or orange reduced males (stock 19). Results in the segregating generation (from females Oo^1Rr virgin or mated to oR or to or males) are given in Table 1 in which are also included three fraternities from F_1 females which had mated to their ivory reduced, o^1r, brothers.

Disregarding for the moment the 21 freaks which are designated by numbers at the right of the table, it may be seen that offspring fall into the expected classes on basis of independent segregation of ivory and reduced and dominance of orange over ivory. Males segregate in all cases while females segregate only when their fathers are recessive. None of the regular males are orange while ivory is concealed in all females except those with ivory fathers. Females segregating into the four classes are summarized as are all regular males at bottom of table. Reduced fall below expectation both in males, 47.7 per cent., and in females, 45.9 per cent. Recombinations have taken place in 50.1 per cent. of males and in 47.9 per cent. of females segregating for both factors. There is thus no evidence of linkage. In order to determine whether there might be linkage at some period during the mother's life or some effect of age of mother upon recombination of factors, segregating progenies of all cultures were added according to vials through which mothers were passed. No significant difference from average was found.

The infrequent types, "freaks," may now be considered.

Biparental Males.

Biparental males, formerly called anomalous or, because they are to be distinguished from other males by possessing paternal traits, patroclinous males, resemble their sisters in showing dominant characters inherited from either or both parents (Whiting, Anna R., in press). They are almost or quite sterile. Their few

daughters tend toward morphological abnormality and are usually sterile.

The nine males classed as biparentals in Table I are distinguished from the regular males by possessing orange eyes of paternal origin. They occur only in bisexual fraternities and only from vials containing females, in other words, before their mother's supply of sperm has become exhausted. Freaks 293 and 293*a* were not tested but are probably biparental. The remaining seven were almost or quite sterile. The four with long-winged father had long wings as expected. Of the five with reduced father, only one (290) had long wings received from its Rr mother.

Mutant and Mosaic Males.

Mutant Male 289.—An F_1 female from ivory reduced female by type male produced in addition to males,—type 75, ivory 61, reduced 69 and ivory reduced 60, an orange reduced male (289) (in vial *f*) with compound eyes rather dark and ocelli of typical orange color. He was mated with eight different females over a period of forty-four days producing daughters by each of them. A subsequent mating after ten days resulted in males only (58) indicating sterility due to age. Of the eight females producing daughters, two, orange reduced, produced 60 orange reduced daughters; five, ivory reduced, produced 172 orange reduced daughters, and one, type carrying reduced, produced 13 type and 4 reduced daughters. The eight mothers also produced 160 normal matroclinous males. Nine of the orange reduced daughters with orange reduced mothers were tested and produced only orange reduced offspring. Freak 289 therefore bred as he appeared somatically transmitting reduced in at least 236 cases and non-black (orange or ivory) in at least 232 cases of which at least 181 were certainly orange.

Mutant Male 319.—An F_1 female from ivory reduced female by type male produced in addition to males,—type 60, ivory 75, reduced 64 and ivory reduced 92, an orange reduced male (319) (in vial *g*) with compound eyes rather dark and ocelli typical for orange. He was mated over a period of fourteen days to eleven ivory reduced females. There were produced 490 ivory reduced males, 2 orange reduced (biparental) males, and 640 orange re-

duced females. Two subsequent matings resulted in males only, 207. Nine orange reduced daughters produced 218 orange reduced males and 210 ivory reduced males. No new types appeared.

Mutant Male 308.—Freak 305 (see below) had eyes which appeared mosaic for black and orange, gonads mosaic for black and ivory. All of his offspring had long wings. Four of his type (di-heterozygous Oo^{i}Rr) daughters from an ivory reduced mother (two had mated to ivory reduced brothers) produced males—type 129, ivory 139, reduced 134, and ivory reduced 121 and females—type 37, ivory 29, reduced 20, and ivory reduced 19. In one of these four fraternities which included females in vials *a*, *b*, and *c*, but males only in vial *d* there occurred, in vial *d*, an orange (deep red) long male (Freak 308).

Freak 308 was tested by mating to twelve ivory reduced females over a period of fifteen days. There were produced males—ivory reduced 701, orange long (biparental) 13, and females orange long 244. Two of these orange biparental males were tested by mating, twice each at an interval of eight days. Males of maternal type only appeared, 67 and 104 respectively, showing that biparental males from Freak 308 tend like other biparental males to be sterile.

Seven orange virgin daughters of Freak 308 segregated males—orange, ivory, orange reduced and ivory reduced as expected. Selection of two deep red of these "orange" males and crossing with ivory reduced females gave orange males in F_2 that were of the usual variability. No effect of selection immediately following mutation appeared.

Freaks 289, 319, and 308 may be regarded as mutants to orange among the expected four classes of males from Oo^{i}Rr mothers.

Mosaic Male 286.—An F_1 female virgin from type female by ivory reduced male produced in addition to males—type 40, ivory 48, reduced 35, and ivory reduced 31, a black-eyed male with left wing reduced, right long (Freak 286), occurring in vial *d* (Fig. 1). He was set with seven different females over a period of eight days. He made prolonged and vigorous attempts to mate which appeared at times successful. No daughters were produced among 427 sons. Examination showed external genitalia to be abnormal

in the presence of an extra right external clasper, as also a curious chitinous structure suggesting an extra malformed penis (Fig. 2). Sterility of this male may be attributed to his inability to complete copulation. His potential breeding capacity is therefore unknown. It is postulated that the first oöcyte division was equational for Rr and that two oötids containing R and r respectively took part in cleavage.

Mosaic Male 287.—An F_1 female virgin from type female by ivory reduced male produced in addition to males—type 37, ivory 38, reduced 36, and ivory reduced 42, an ivory-eyed male (Freak 287) with right wings reduced, left long, occurring in vial d (Fig. 10). Eyes and ocelli were typical for ivory.

Freak 287 was mated to each of thirteen females over a period of fifty-two days. Daughters were produced by all of them. Three subsequent matings resulted in males only, 276, indicating sterility due to age. In addition to the 270 males of maternal type produced by the first thirteen females there were the following biparental offspring:

Six ivory reduced females produced 113 black reduced, 8 males and 105 females.

Three orange/ivory reduced females produced 120 black reduced, 5 males and 115 females, and 1 ivory long female.

Four homozygous orange reduced females produced 26 black reduced, 4 males and 22 females.

One homozygous ivory long female produced 24 type, 2 males and 22 females.

Freak 287 therefore had the capacity to produce biparental males, 19 to 265 females. Biparental males from recessive mothers indicate as do females what genetic factors are in the sperm. Black biparental offspring number 284 of which those with reduced mothers, 260, were all reduced. The single ivory long daughter from orange/ivory reduced mother represents the combination visible in the eyes and left wing of Freak 287.

Each of two of the reduced biparental males with ivory reduced mother was mated once. Ivory reduced males only resulted, indicating that biparental sons of mosaic males tend, like other biparental males, to be sterile.

The origin of Freak 287 may be expressed

First polar body	$Oo^{1}Rr$
Cleavage nuclei	*Or* $o^{1}R$

Italics are used to designate origin of gonads.

Mosaic Male 307.—Five type females, F_1 from ivory female (stock 17) by type male (stock 24) produced males only—type 412 and ivory 408 and in vial h a male (Freak 307) with pale orange eyes and ocelli typical for orange. He was mated with fourteen ivory females over a period of twenty-five days. Besides the 939 ivory sons there were 470 biparentals, all black, 9 males and 461 females.

Two of these biparental males were tested by mating, each to two females at an interval of eight days. Males only resulted, 66 and 104 respectively. Biparental males from Freak 307 are therefore of the usual sterile type.

Three daughters of Freak 307, produced males only, type 139, ivory 154.

Reduced is not concerned in the production of Freak 307. His origin may be expressed

First polar body	Oo^{1}
Cleavage nuclei	*O* o^{1}

If it be assumed that o^{1} mutated to o, Freak 307 is not only a mosaic but also a mutant. This question is discussed below.

Mosaic Male 321.—A black reduced male was mated to an ivory reduced female. 2 ivory reduced males and 28 black reduced females resulted. One of these daughters produced ivory reduced males 2, and a reduced male (321) with ocelli and left eye black, right eye black with red or orange area ventrally (Fig. 9). Long wings are not involved in this experiment. Freak 321 was mated to twenty-five ivory females over a period of twelve days. Ivory males 1816, black (biparental) males 66, and black females 806, resulted.

Freak 321 may be regarded either as a mosaic of black and ivory in which the ivory has taken on a reddish appearance due to proximity of "black" tissue or as a simultaneous mosaic and mutant. Its origin may be from a binucleate egg, or from a normal egg with subsequent mutation of O to o.

Mosaic Male 323.—A type male was mated with an ivory re-

duced female. Type females only (26) resulted. One of these produced 44 males—type, ivory, reduced, ivory reduced as expected and in vial *d* a male, Freak 323, with long wings and mosaic eyes and ocelli (Figs. 5 and 6). He was mated with sixteen ivory reduced females over a period of eight days. Ivory reduced males 158, type (biparental) males 16, and type females 280, resulted.

Freak 323 is comparable with Freak 321 in that light areas of eyes are orange rather than ivory. They are, however, much more extensive. His origin on basis of two oötids may be expressed:

First polar body	$Oo^{i}rr$
Cleavage nuclei	$OR \mid o^{i}R$

o^{i} may have mutated to o or the orange color may be purely somatic.

Mosaic Male 283.—An F_1 female from ivory reduced female by type male produced in addition to type males 50, ivory males 36, reduced males 38, and ivory reduced males 41, an orange-eyed male (Freak 283) with long wings occurring in vial *c*.

Two matings of Freak 283 with ivory long females (stock 17) resulted in 133 ivory males and 161 type females. The production of black-eyed daughters indicates that Freak 283 was a mosaic involving black. Fifteen of these type daughters isolated as virgin produced males—type 321, ivory 277, reduced 252, and ivory reduced 278. The production of reduced males proves that Freak 283 transmitted reduced and was therefore mosaic of long and reduced, as well as of black and ivory (or orange).

A mating of Freak 283 with orange reduced (stock 19) female resulted in orange reduced males 41, reduced females 56, showing directly that Freak 283 produced reduced only. Five of these reduced females produced reduced males 238, and orange reduced males 208, as expected. Freak 283 was also mated to type female (stock 1). This resulted in type males 93, and type females 30. Six of these females produced males—type 99 and reduced 83, and one which was mated to Freak 283 produced females—type 14, and reduced 15.

Freak 283 was also mated with three ivory reduced females. There resulted ivory reduced males 28, and reduced females 139, as well as a gynandromorph (Freak 304) with reduced wings and

eyes mosaic of black and orange discussed below. Eight of these females which had mated with their ivory reduced brothers produced males—reduced 30, ivory reduced 34, and females—reduced 93, and ivory reduced 92, as expected.

Summarizing the results of the eight matings of Freak 283 extending over a period of fourteen days, we find that he transmitted black, O, in at least 363 cases and reduced, r, in at least 232 cases. In other words he bred as a black reduced and no new factors or unusual types appeared except the gynandromorph, 304, discussed below. His origin on the basis of egg binuclearity may be expressed.

First polar body	$Oo^{1}Rr$
Cleavage nuclei	$Or \mid o^{1}R$

Freaks 283 and 307 differ from freaks 321 and 323, in that there is no trace of black in the eyes. Orange appearance is therefore not due to proximity of black facets, but to some physiological influence from "black" tissue or to mutation.

Mosaic Male 306.—An F_1 female from type female by ivory reduced male was mated with an orange long male (stock 12). From vials *a–d* there appeared males—type 9, ivory 13, reduced 6, ivory reduced 9, and females—type 26, and orange 27, as expected. Subsequently (vials *e–h*) there appeared males—type 26, ivory 26, reduced 34, ivory reduced 17, and a long-winged male with light orange eyes and mosaic ocelli appearing in vial *h* (Freak 306, Figs. 7 and 8). As may be seen from the figure the right ocellus is without pigment, even the slight amount characteristic of "orange" being absent. Anterior and possibly also left ocelli are mosaic. That the thorax is also possibly mosaic is indicated by its lighter color on right side.

Freak 306 was mated over a period of thirty-eight days with nine ivory reduced females and with five ivory long carrying reduced. In addition to the 814 males of maternal type there were 601 ivory long daughters. 464 of these (from ivory reduced mothers) prove that Freak 306 transmitted long. Of the remainder from Rr mothers, about half must have been long because of factor R in the sperm.

Freak 306 therefore breeds like an ivory long. The ocelli cer-

tainly contain black and either ivory or orange. The compound eyes are of definite orange appearance. Origin may be expressed

First polar body	$Oo^{1}rr$
Cleavage nuclei	$OR \mid o^{1}R$

"Black" tissue is regarded as present because of the ocelli. If orange appearance be due to mutation of o^{1} to o then there are three types of tissue here, O, o, and o^{1}.

Mosaic Male 305.—An F_1 female from type female by ivory reduced male was mated with an orange long male (stock 3). There were produced males—type 23, ivory 24, reduced 18, and ivory reduced 28, and females—type 94, and orange 74 as expected. There also appeared in vial *g* along with type and orange sisters, a male, Freak 305, with long wings, orange ocelli, and eyes mosaic of black and orange (Figs. 11 and 12).

Freak 305 was mated with eleven females over a period of twenty-nine days. Three subsequent matings resulted in males only, 297, indicating sterility due to age. The eleven females producing daughters gave in addition to 529 matroclinous sons, the following biparentals:

Three homozygous ivory females produced type females 6, ivory females, 122.

Eight ivory reduced females produced type females 19, ivory long male 1, ivory long females 243, ivory females with wings unexpanded 8.

Freak 305 therefore transmits long wings, 263 cases, both in association with black, 19 cases, and ivory, 244 cases.

Table 2 shows distribution of the 25 black and 374 ivory progeny according to age of Freak 305.

Origin of Freak 305 may be expressed

First polar body	$Oo^{1}rr$
Cleavage nuclei	$OR \mid o^{1}R$

Gonads are evidently mosaic for O and o^{1}, eyes for O and o^{1} or O and o.

Diploid Mosaics.

Mosaic Female 313.—An orange long female (stock 10) mated with an ivory reduced male (stock 20) produced orange long—

males 20 and females 6 and an orange female (Freak 313) with left wing reduced, right long (Figs. 22 and 23) and fifteen joints in each antenna, typical for female.

TABLE II.

DISTRIBUTION OF BLACK AND IVORY PROGENY ACCORDING TO AGE OF MOSAIC MALE 305.

Progeny.	Age in Days since Eclosion.											Total.
	4	5	7	12	13	16	18	26	28	30	32	
Black.....		4		1	5	2	2	1	10			25
Ivory.....	9	56	10	49	64	48	39	23	74	1	1	374

Freak 313 which had mated with her orange brothers produced males—orange 13, ivory 9, orange reduced 13, ivory reduced 10, and females—orange 39. Two of these females, when tested, produced reduced sons while five gave only long. Eggs of Freak 313 bearing R therefore numbered 27 while eggs bearing r numbered 25.

Freak 313 may be regarded as a heterozygous female, $oo^{1}Rr$, in which some somatic mitotic irregularity occurred eliminating R in the development of the left wing.

It may be noted that this mosaic female, unlike the mosaic males thus far discussed, had a homozygous mother.

Mosaic Biparental Male 318.—An ivory reduced female mated with an ivory long male produced ivory reduced males, ivory long (biparental) males, and ivory long females and ivory male (Freak 318) with left wing reduced, right wing long and terminal joints of right antenna fused (Figs. 3 and 4).

Freak 318 differs from mosaic males previously described in having a homozygous mother. It is evidently biparental, receiving R from its father. Its mosaicism was regarded as comparable in origin to that of female, 313 (somatic mitotic irregularity eliminating R). Being biparental, it was expected to be sterile or to produce a few sterile daughters. It was mated with six females over a period of twelve days. Nothing but males of maternal type appeared, totalling 946.

GYNANDROMORPHS.

Gynandromorph 296.—An F_1 female from type female by ivory reduced male was mated with an orange reduced male (stock 19). There were produced males—type 23, ivory 29, reduced 28, ivory reduced 18, and females—type 34, orange 25, reduced 26, orange reduced 35, and in vial *c* a gynandromorph (Freak 296) with ivory eyes, right wing long, left reduced, sixteen joints in right antenna, 24 in left, and female abdomen (Figs. 18, 21). Measurements showed ocelli of male type. Responses resembled those of the male.

Since the father was orange the male parts (head with ivory ocelli and compound eyes) were of maternal origin. Difference in primaries indicates that one oötid contained R, the other r.

Gynandromorph 302.—An F_1 female from type female by ivory reduced male was mated to an orange reduced male (stock 19). There were produced males—type 14, ivory 17, reduced 10, ivory reduced 11, and females—type 39, orange 32, reduced 33, orange reduced 34, and in vial *f* a gynandromorph (Freak 302) with black eyes and ocelli, male head (right antenna with 20 joints, left with 21 and ocelli of male size), long wings and female abdomen. Responses were characteristic of the male.

Head and wings show maternal traits. Antennæ and ocelli and therefore presumably compound eyes are male. Whether wings are male like head (haploid) or female like abdomen (diploid) is uncertain.

Gynandromorph 303.—An F_1 female from type female by ivory reduced male was mated to an orange long male (stock 12). There were produced males—type 10, ivory 14, reduced 11, and ivory reduced 8 and females—type 36, and orange 46, and a gynandromorph (Freak 303) in vial *b* with ivory eyes and ocelli, long wings, male head, antennæ with 23 joints) and female abdomen (Fig. 24). Responses were characteristic of the male.

Eye color indicates maternal origin of male parts (head). Wing character may have been derived from either parent and may be either haploid (male) or diploid (female).

Gynandromorph 288.—An ivory reduced female mated to a light (black eyes, dilute ocelli), o^1, long male produced 4 ivory re-

duced males, 3 light long males, (biparentals), 23 light long females and a gynandromorph (Freak 288) in vial *c* with ivory eyes, male head (23 joints in each antenna), left wing long, right reduced, and abdomen female (Figs. 19 and 20). Pupal membrane adherred to tip of abdomen and specimen was nearly dead when found, perhaps diseased. Darker color of right side of sternum indicated that right side of thorax, with wings reduced (matroclinous) was probably male. This is consistent with fact that head, also matroclinous, is male.

Gynandromorph 322.—An ivory reduced female mated to a black reduced male produced 10 ivory reduced males, 46 black reduced females and in vial *b* an ivory reduced gynandromorph (Freak 322) with male head (20 joints in each antenna) and a female abdomen. Reactions were male but weak. Long wings are not concerned in the cross. Eye color shows male parts to be maternal in origin.

Gynandromorph 304.—An ivory reduced female mated to Freak 283, orange long male breeding as black reduced (see above) produced ivory reduced males 12, black reduced females 71, and in vial *b* a gynandromorph (Freak 304) with reduced wings and eyes mosaic for black and orange (Figs. 13–17). Left antenna is clearly female (15 joints), right is male but with terminal joints fused. Ocelli are mosaic, the right and anterior with considerable black pigment, predominantly female; the left with very little pigment clearly larger than the right and exclusively or predominantly male. Compound eyes are mosaic for black and orange. Abdomen is entirely female, sting split, and right gonapophysis, in agreement with right antenna, deficient. The left secondary is considerably longer than the right indicating femaleness.

Responses proved to be exclusively male. Repeated and vigorous attempts were made to mate with females.

Freak 304 is evidently an intricate mosaic of male and female parts, very different in this respect from any gynandromorphs previously found in this species.

The size of the left ocellus indicates that it is male and its reduced amount of pigment shows its maternal origin. Black regions of eyes indicate paternal influence and are assumed to be female (diploid). Orange regions of eyes are presumably male

(haploid) and of maternal origin, ivory being changed to orange at least in appearance.

Gynandromorph 325.—An ivory reduced female mated to a black reduced male produced 7 black reduced females and in vial *a* a small-sized gynandromorph (Freak 322) with male head and female abdomen. Eyes and ocelli were entirely black, the latter of male size. Wings were wrinkled with skin adhering. Abdomen was collapsed, genitalia immature. Sternites were of female type. Antennæ were deficient. The left had seventeen joints, the right eighteen with the two terminal fused. These antennæ are interpreted as male since counts of joints of over eighteen hundred female antennæ previously made showed none above sixteen and this number occurred in the larger individuals only.

The specimen was weak when found. Reactions could not be tested.

Freak 322 is the first gynandromorph found with male parts patroclinous. Male biparentalism is indicated by the general abnormality and weakness of the specimen. As explanation for origin, either egg binuclearity and dispermy or somatic mitotic irregularity may be suggested.

Frequency of Occurrence of Mutants and Mosaics.

Of the 14,023 wasps recorded in Table I., 9,787 (normal males 9,780, mutant males 3, and mosaic males 4) may be regarded as coming from unfertilized eggs and 4,236 (normal females 4,224, biparental males 9, and gynandromorphs 3) from fertilized eggs laid by diheterozygous mothers, $Oo^{l}Rr$. These mothers came in all cases from crosses of stock 20, ivory reduced, and a type stock, either 11 or 24.

Mosaic and mutant males and gynandromorphs number 9 among the 7,908 descended from stock 11, while there is but one mosaic male among the 6,115 descendents of stock 24. This may indicate a difference in hereditary tendency toward binuclearity in favor of stock 11.

Among the eleven haploid freak males above discussed, three (289, 308, 319) were mutants but not obviously mosaics, two (286, 287) were mosaics but not obviously mutants, and six (283, 305, 306, 307, 321, 323) were mosaics and at least apparently

mutants for they showed orange color in eyes. Since two (283, 307) at least of the latter would not have been tested and detected as mosaics had they not been orange, it was thought that other males apparently segregating in normal manner might be mosaic also. Gonads might differ from soma.

Accordingly F_2 males of normal appearance from a cross of ivory reduced (20) by type (11) were tested by mating to ivory reduced females. Thirty-five type bred as type (offspring totaling ivory reduced males 172, type males 24, type females 750), forty-two ivory bred as ivory (offspring totalling ivory reduced males 218, ivory males 28, ivory females 911), thirty-eight reduced bred as reduced (offspring totalling ivory reduced males 254, reduced males 21, reduced females 1,217) and twenty-nine ivory reduced bred as ivory reduced (offspring totalling 77 males and 285 females. Thus of 144 normally segregating males from $Oo^{i}Rr$ females none showed gonads different from soma.

Hypotheses Suggested.

The data above presented show that orange eye color may appear in males from eggs laid by mothers heterozygous for black and ivory, Oo^{i}, in at least two or possibly three different ways. Biparental males obviously receive the factor O from their father. Males with eyes mosaic for black and orange have thus far bred as black (321, 323), as ivory (306), or as black and ivory mosaic (305). In the case of three of these it may be supposed that proximity of black facets may cause the "ivory" to take on an orange appearance, but number 306 had compound eyes entirely orange with dark pigment appearing in the ocelli only. Moreover since two orange males (283, 470), breeding as black showed no trace of black pigment, it must be supposed that influence of "black" tissue in changing ivory to orange is not merely an optical effect from proximity of black facets, but is due to some physiological (enzymatic?) influence which actually transforms ivory to orange. If this be the case, it must be supposed that in number 287 (ivory breeding as black-ivory mosaic) the "black" tissue did not involve the "enzyme-producing gland."

An alternative to this "somatic" explanation is to suppose that actual genetic change has taken place. In the mosaic males gonads

have not included this orange tissue. It may be suggested that the three orange mutants (289, 308, 319), although not obviously mosaics, may have been derived from binucleate eggs carrying O and o^1 for their mothers were Oo^1.

The hypothesis of mutation of o^1 to o caused by association with O tissue brings the various phenomena into harmony. Evidence both that it is o^1 rather than O which has changed and that this change has taken place in somatogenesis rather than in ovogenesis is afforded by the mosaic-eyed (black-orange) gynandromorph 304 in which O must have been derived from the male parent for the mother was o^1o^1.

Evidence decisive between the somatic and the genetic hypotheses has not yet appeared. A mosaic male from Oo^1 female producing o gametes or a male with eyes mosaic of orange and ivory would be of much interest in this connection.

Summary.

1. Independent segregation of ivory (eye color) and reduced (wings) is shown in progeny of Oo^1Rr females.

2. When such females are mated to orange, o, males there occur among the normal offspring, orange (biparental) males with characteristic sterility.

3. One female from RR female by r male and one sterile biparental male from rr female by R male were each characterized by possessing one long and one reduced wing. Elimination of chromosome bearing R is suggested as explanation.

4. Eight mosaic males were produced by Oo^1 females. Of these—one was sterile, four produced black daughters only, one ivory only, and two both black and ivory. It is suggested that these males arise from binucleate eggs and that gynandromorphs are produced when one nucleus of such eggs is fertilized.

5. Of seven gynandromorphs six showed male parts matroclinous while in one they appeared patroclinous. In the latter case they may have been diploid, comparable to ordinary male biparentalism.

6. Two mosaic males had eyes completely orange while four mosaic males and one gynandromorph had eyes mosaic of black and orange. Since presumably only black or ivory entered into

the gametes producing these mosaics, the orange color must have been due to some somatic physiological effect or to mutation.

7. Three mutant orange males showing no mosaicism and breeding as orange were produced by $Oo^{1}Rr$ females.

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EXPLANATION OF PLATE I.

FIGS. 1–24. Camera lucida drawings of various parts of mosaics of *Habrobracon juglandis*.

Figs. 1, 4, 10, 13, 18, 19, 22. × 10.

Figs. 3, 11, 12, 14, 15, 17, 20, 21, 23, 24. × 22.

Figs. 2, 6, 9. × 30.

Figs. 5, 7, 8, 16. × 63.

Figs. 1–12 are from mosaic males.

Figs. 13–21 and 24 are from gynandromorphs.

Figs. 22 and 23 are from a mosaic female.

Figs. 1 and 2, wings and ventral view of genitalia of mosaic male 286.

Figs. 3 and 4, right antennæ and wings of mosaic male 318.

Figs. 5 and 6, dorsal view of ocelli and anterior view of head of mosaic-eyed male 323.

Figs. 7 and 8, dorsal and anterior view of ocelli of mosaic male 306.

Fig. 9, right view of head of mosaic-eyed male 321.

Fig. 10, wings of mosaic male 287.

Figs. 11 and 12, dorsal and ventral views of head of mosaic-eyed male 305.

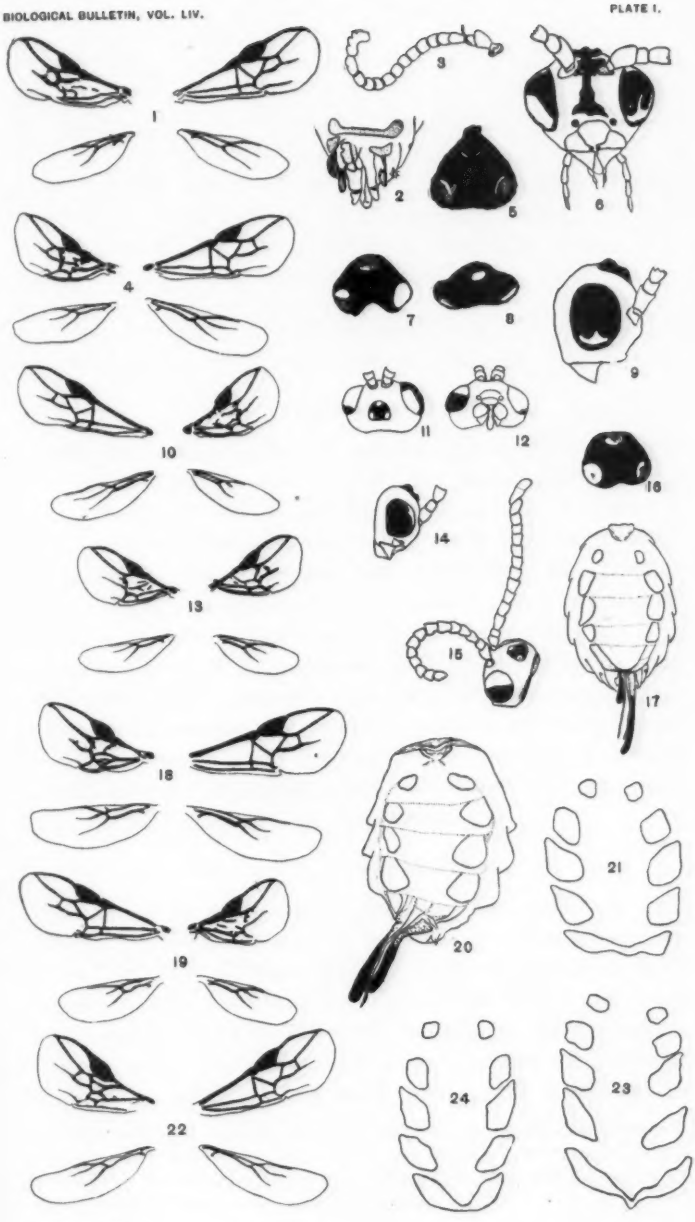
Fig. 13, wings, Figs. 14 and 15, right and dorso-sinistral views of head. Fig. 16, dorsal view of ocelli and Fig. 17, ventral view of abdomen of mosaic-eyed gynandromorph 304.

Figs. 18 and 21, wings and abdominal sternites of gynandromorph 296.

Figs. 19 and 20, wings and ventral view of abdomen of gynandromorph 288.

Figs. 22 and 23, wings and abdominal sternites of mosaic female 313.

Fig. 24, abdominal sternites of gynandromorph 303.



P. W. WHITING.

OVULATION IN THE FOUR-TOED SALAMANDER, *HEMIDACTYLIUM SCUTATUM*, AND THE EXTERNAL FEATURES OF CLEAVAGE AND GASTRULATION.

R. R. HUMPHREY,

DEPARTMENT OF ANATOMY, SCHOOL OF MEDICINE, UNIVERSITY OF BUFFALO.

INTRODUCTION.

Incidental to the collection of material for a study on the germ cells of *Hemidactylium scutatum* the writer brought to the laboratory a female of this species which subsequently deposited a number of ova while under observation. The eggs laid proving fertile, the progress of cleavage and gastrulation was carefully noted. Though the observations made cover but a very limited material, they serve to supplement the work of Bishop ('18) who begins his account of the development of Hemidactylium with the neural plate stage; moreover, ovulation in this species seems not to have been previously observed and reported. For these reasons the observations made are here reported, even though the limited material prevents consideration of but little more than the superficial features of cleavage and gastrulation.

OVULATION IN *Hemidactylium*.

The female on which these observations were made was captured in the sphagnum bog on the shores of Mud Pond near Ithaca,[1] the site of Bishop's discovery of the egg-laying habits of this species some years before. This particlar female was found in a sphagnum hillock together with two eggs, on the morning of May 11, 1923. She was brought to the laboratory the afternoon of that day, and placed in a small glass dish of sphagnum, to-

[1] This study was carried on in the laboratories of the Department of Histology and Embryology of Cornell University. The writer wishes to acknowledge the kindness of Dr. B. F. Kingsbury at whose suggestion the observations herein reported were undertaken.

gether with the eggs assumed to be hers. About 9 P.M. of the same day it was noted that eight eggs were present, all but three of which were scattered over the bottom of the dish. The female had been partially concealed by the sphagnum, and was of course necessarily disturbed by the attempt to locate her and her eggs for observation. It would seem, however, that her position when first accurately observed was probably but little changed, since she was in contact with two ova which later observations would indicate had been but recently extruded. She lay with her back turned against the bottom of the dish, her abdomen upturned against the overlying sphagnum. Two eggs lay above her, still in contact with her ventral body wall immediately cephalad of the cloacal opening, but adherent to the overlying sphagnum; by gently pulling upon this sphagnum the eggs could readily be pulled away from the body of the female, who was not at all disturbed by this procedure, but remained quietly in position. Her body was somewhat curved, so that the middle part of her abdomen was in contact with the side of the dish; the end of her tail also lay in contact with the side of the dish, elevated along it and not in contact with the bottom.

With the body of the female in this position, it was noted at 9:14 P.M. that an egg was beginning to appear at the cloacal opening. The female made no unusual movements, but lay quietly while the egg continued to move slowly outward. In about two or three minutes it was practically expelled from the cloaca, but the female lay motionless for several minutes before a slight shift of her body left the egg attached to the overlying sphagnum.

The process of ovulation was not repeated until about 10:55 P.M. During the expulsion of this second egg the female twisted the front half of her body in such fashion that her left side was toward the bottom of the dish; the caudal part of the body however, was kept in its former position, with the dorsum against the bottom of the dish and the vent towards the overlying sphagnum. This position was retained for several minutes following the expulsion of the egg. After about twelve minutes the female was brought into a brighter light for more careful observations, and thereupon moved slowly away from the position she had maintained throughout the expulsion of the two ova. She now lay

with the abdomen pressed against the side of the dish, the cephalic third of her body in a vertical position, the remainder, except for the end of the tail, extending in a horizontal direction along the junction of the side and bottom of the dish. In this position a third egg was expelled. As in previous ovulations, no unusual movements of the female were noted during the process. Preceding the appearance of the egg at the cloacal opening the female was seen to straighten her body and move the hind limbs slightly—movements perhaps necessary to facilitate the passage of the egg through the pelvis. The female then lay motionless with the abdomen pressed against the side of the dish while the egg was slowly forced from the cloacal orifice and against the glass. After the female had remained for about eight minutes with the egg in this position she was gently pushed away from the side of the dish, to which the egg remained adherent. As she showed no indication of further attempts at ovulation, the observations were discontinued. This female subsequently deposited a few other ova, but at times when she was not under observation.

The position of the female during the expulsion of the first two ova is of considerable interest, since it may possibly represent an approach to the normal. A horizontal position of the body with the ventral surface uppermost at the beginning of ovulation would prevent the eggs from dropping down into the spaces of the sphagnum, and would insure the contact and adherence of eggs successively expelled from the cloaca, since each egg as it leaves the cloacal opening would be supported upon the body of the female in contact with the egg previously extruded. With the accumulation of a number of eggs, the size of the egg mass, together with the viscid character of its surface would effectively prevent its dropping downward in the spaces of the sphagnum. Females taken in sphagnum bogs after ovulation ordinarily have their eggs loosely arranged in a mass around or over which the body of the animal is coiled.

The eggs when first expelled from the cloaca show only an extremely thin gelatinuous capsule; after several minutes this is noticeably thicker, due to the absorption of water from the moist environment. The outermost layer of this jelly is of a semi-fluid, viscid nature, which causes the eggs to adhere readily to each

other or to sphagnum, glass, or other gelatinous egg envelopes, while a thin, membrane (vitelline membrane) closely surrounds the egg or embryo and separates it from the more fluid gelatinous envelope; a similar membrane of the egg of *Cryptobranchus* is stated by Smith ('26) to be identical with the zona pellucida of ovarian eggs.

Fertilization in this species is internal, as in other Urodeles. The breeding habits of the animals are quite unknown to the writer, but since males have not been found with the females near ponds at the time of ovulation (Bishop, '18; Blanchard, '23) it may be concluded that mating occurs at some previous time, and very probably in a terrestrial environment, the transfer of sperm from male to female possibly being effected by contact of the ventral surfaces of the animals. Bishop quotes C. and H. Thompson ('12) and Moesel ('18) to the effect that males and females have been taken from beneath the same log or stone during the spring months.

The female possesses the sperm-storing organ or spermatheca characteristic of Urodeles. According to Dieckmann ('27) the spermatheca of this species is unique in that it consists of but a small number of spermathecal tubules opening directly into the cloacal chamber; the latter feature is regarded as a primitive one, while the former (reduction of tubules) is characteristic of the most highly developed spermathecæ, in which the tubules open to a single duct-like tube (common tube,—a modified portion of the cloacal chamber). Dieckmann's descriptions of the cloaca and spermatheca of this species are based upon two specimens captured in August near Buffalo, New York. In one of these, spermatozoa are very abundant, in the other absent; on the assumption that *Hemidactylium* males are incapable of mating during the summer months, as are practically all Urodeles, these spermatozoa must be interpreted as having been stored for at least several weeks after a mating in the spring months. Their large number and whorl-like arrangement in the spermatheca suggest however, that no ovulation had occurred after they had been received into the spermathecal tubules.

Cleavage.

The eggs laid under observation in the laboratory, together with others deposited by the same female, proved to be fertile, making possible a study of the early cleavage divisions. While the limited number of eggs necessarily prevented fixation and sectioning of successive cleavage stages, the external features of segmentation were carefully noted over a period of several hours, or until gastrulation was in progress.

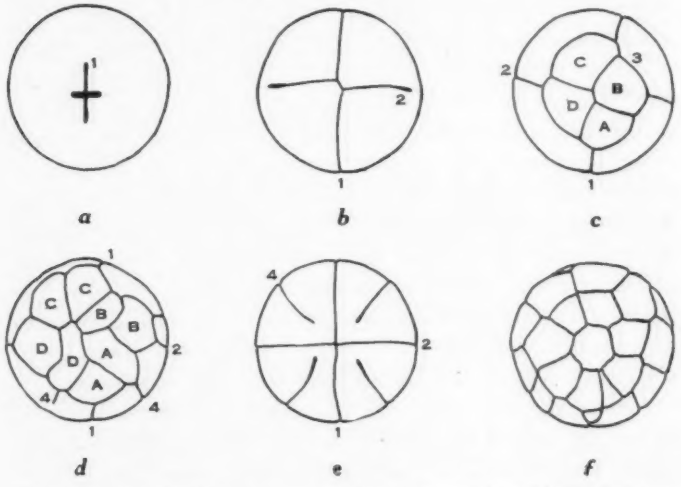
In order to follow the progress of cleavage in individual eggs the ova were removed singly to numbered Syracuse watch glasses each containing a few drops of water; each egg was left in contact with a small bit of sphagnum rather than in the water. The watch glasses were transferred to the stage of a binocular microscope for observations; the position of the egg was changed as desired, either by moving the sphagnum to which it was attached, or by direct manipulation with needles. Though it was realized desirable to handle the eggs as little as possible, the difficulty of keeping them in position for viewing the vegetal hemisphere necessitated considerable manipulation. Though possibly this and other environmental factors may have contributed to certain of the atypical features of cleavage observed in some of the ova, it is nevertheless certain that eggs showing such features in some cases gave rise to normal embryos. The eggs were of course kept under observation without removal of their protective membranes.

Some of the eggs, when first viewed under the microscope, still showed the polar bodies adherent to their surfaces; these as a rule became indistinguishable after the first two or three cleavage divisions had been completed. The stage of maturation at the time of laying was not determined.

Since in none of the eggs laid in the laboratory during the night of May 11–12 did the first cleavage furrow make its appearance later than 10: 30 A.M. of the following day, cleavage may be assumed to begin within from ten to fifteen hours after the egg is deposited. The egg of *Hemidactylium* apparently agrees with that of *Spelerpes* (*Eurycea*) in this respect (see Goodale, '11).

The pattern of cleavage will first be described in an egg in which the divisions proceeded in a relatively orderly, symmetrical fashion. In this egg the first cleavage furrow was first noted at

9:30 A.M.; it then extended over about a sixth of the egg circumference (see Fig. 1, *a*). From its middle point at the animal pole a short broad furrow extended at right angles to it on either side, the two furrows together forming a rather distinct cross. The short transverse furrow subsequently disappeared, being practically invisible by the time the first cleavage furrow had reached the egg equator. A similar furrow was observed in only one other egg. In another several short furrows radiating from the animal pole were formed preceding the appearance of the first cleavage furrow and disappeared after the latter had extended over a fourth of the egg circumference.



a *b* *c*

d *e* *f*

FIG. 1. Egg showing the most regular or symmetrical cleavage pattern observed. Viewed from animal pole except in *e;* cleavage furrows as numbered. *a*, 9:30 A.M. 5–12–23; *b*, 12 M.; *c*, 2 P.M.; *d*, 3:40 P.M.; *e*, vegetal hemisphere at 5 P.M.; *f*, 8:30 P.M. The origin of the micromeres in *d* is indicated by the lettering.

The progress of the first cleavage furrow through the more heavily-yolked vegetal hemisphere of the egg was at a much slower rate than its formation between animal pole and equator. From this egg and others observed we may conclude that the furrow is usually completed (on the egg surface) within about two hours after its first appearance.

The second cleavage furrows made their appearance before the first had been completed. In Fig. 1, *b* they are shown extending practically to the equator of the egg, 2½ hours later than the stage of Fig. 1, *a*. The first cleavage furrow in this egg is now complete. The second cleavage furrow apparently reaches the vegetal pole of the egg in from two to three hours.

The third set of cleavage furrows appeared about an hour after the stage of Fig. 1, *b*, and were completed within an hour, the egg at 2 P.M. having the appearance shown in Fig. 1, *c*. These furrows were roughly parallel with the egg equator and lay well up in the animal hemisphere. The four micromeres thus formed rotated a short distance in a clockwise direction to the position they occupy in Fig. 1, *c*.

The cleavage furrows of the fourth set were completed in the micromeres at 3:40 P.M., though just beginning to appear at that time in the macromeres. The direction of these furrows in *B* and *D* was roughly parallel with the first cleavage furrow; in *A* it was at right angles to this furrow, and in *C* it was meridional. With the completion of the division the daughter micromeres underwent readjustments of form and position such that the egg at 3:40 P.M. presented the appearance of Fig. 1, *d*. The fourth cleavage furrows of the macromeres are shown in this figure at an early stage of their formation; they had not been completed when the egg was sketched at 5 P.M. (Fig. 1, *e*). Their direction, while approximately meridional, was such that they would end in contact with the second cleavage furrow a short distance from the vegetal pole.

No further observations on this egg were made until 8:30 P.M., at which time it presented the appearance indicated in Fig. 1, *f*. It was impossible at this time to determine accurately the relation of any of the micromeres to those of Fig. 1, *d*.

It would appear from the above that cleavage in *Hemidactylium* is similar to that in the egg of *Eurycea* (Goodale) and is of the holoblastic unequal type characteristic of the moderately-yolked eggs of Urodeles. As in *Eurycea* and other species, however, the cleavage divisions conform to a symmetrical pattern for but few divisions if at all, and the blastomeres soon become of such irregular form and arrangement that their descent from any particular cell of the four- or eight-celled stage can not be determined.

Though irregularity of the cleavage pattern may be expected to appear during the third or fourth division in any egg of this species, as shown in Fig. 1, *d*, it is of earlier origin in a great many cases. Of ten eggs in which the furrow pattern could be determined for a stage equivalent to that of Fig. 1, *b*, in only four did it conform to this plan, and of these only one egg showed in the third division the regularity of pattern illustrated in Fig. 1, *c*.

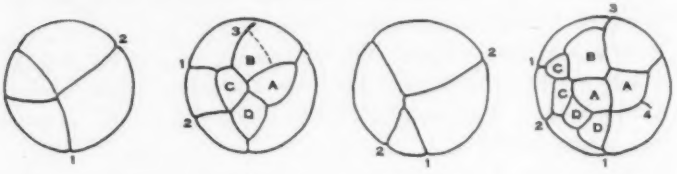


FIG. 2. Egg showing cleavage pattern resulting from inequality in the first division. Viewed from animal pole except in *c;* furrows numbered and origin of cells in *d* shown by lettering. *a*, 1:15 P.M., 5–12–23; *b* and *c*, 3:25 P.M.; *d*, 5 P.M.

Irregularity or asymmetry of cleavage in two of the ten eggs began with the first division. In one case it consisted merely in an inequality in the division of the egg (see Fig. 2, *a*); in the second division the larger of the two cells was divided more slowly than the smaller, the cleavage furrow in it reaching only thirty degrees below the egg equator when division in the smaller cell was completed. The third cleavage furrows were horizontal in three cells; in the fourth the furrow took a meridional direction, as shown in Fig. 2, *b*, in which it reaches almost to the egg equator. The vegetal hemisphere of the egg now appeared as in Fig. 2, *c*. A cleavage furrow along the dotted line of Fig. 2, *b*, subsequently cut off a micromere from cell B; this, with the division of micromeres *A*, *C*, and *D*, formed a group of seven cells of very unequal sizes arranged as in Fig. 2, *d*. The descendents of micromeres *C* and *D* formed a group of cells still distinguishable by their smaller size several hours later, in the fine-celled blastula stage; they then lay just above the egg equator. This egg subsequently completed gastrulation in the normal manner.

In the second egg to show irregularity beginning with the first division, the first furrow to appear had the position illustrated in Fig. 3, *a*. Within two hours other furrows of irregular direction had developed, giving the animal hemisphere the appearance of Fig. 3, *b*. At this time no furrows extended below the equator. Later several furrows extended into the vegetal hemisphere (Fig. 3, *c*). Fig. 3, *d* illustrates the appearance of the animal hemisphere in this egg about eight hours after the beginning of cleavage. Though continuing development for 24 hours longer and reaching a fine-celled blastula stage this egg finally died without beginning gastrulation. Its markedly atypical cleavage, therefore, may well have been the result of injury from drying, handling, or other causes.

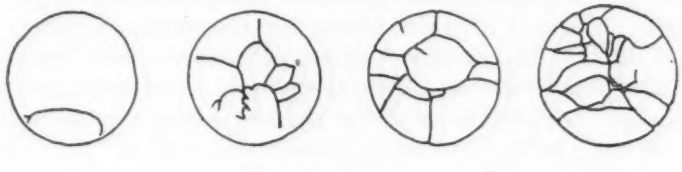


FIG. 3. Egg showing extremely irregular cleavage pattern beginning with the first division. Viewed from animal pole except in *c*. *a*, 2:45 P.M., 5–12–23; *b*, 4:45 P.M.; *c*, 8 P.M.; *d*, 10:25 P.M.

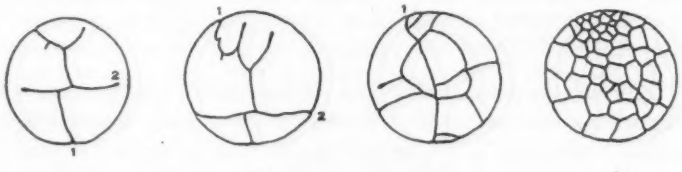


FIG. 4. Egg showing irregular cleavage pattern. Viewed from animal pole; egg in *b* is tilted to show full extent of atypical furrows. *a*, 10:25 A.M., 5–12–23; *b*, 12 M.; *c*, 1:45 P.M.; *d*, 10:45 P.M.

Another egg with almost as pronounced irregularity of cleavage as that illustrated in Fig. 3 nevertheless gave rise to a normal embryo. In this egg the first cleavage furrow developed normally, and had completely encircled the egg within three hours.

Before the usual second furrows made their appearance, a furrow was noted leading from the first a short distance above the egg equator. About a half-hour later the appearance was as in Fig. 4, *a*, with the second furrows now present. Later the egg showed other furrows in the region in which the first atypical one had made its appearance (Fig. 4, *b*) and about five hours after the onset of cleavage the animal hemisphere displayed the atypical pattern of Fig. 4, *c*. In the somewhat later blastula stage illustrated in Fig. 4, *d* this egg showed a group of distinctly smaller micromeres at and below the equator in the region of the first atypical furrow. Though this peculiarity persisted until late in cleavage this egg completed gastrulation in the normal fashion.

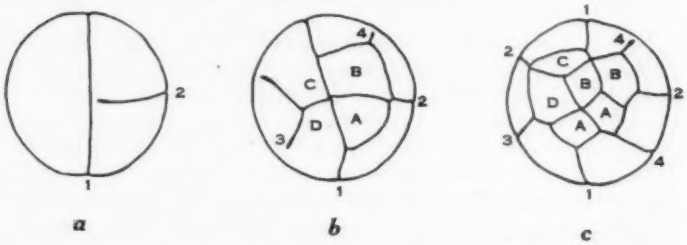


FIG. 5. Egg showing irregular cleavage pattern starting with retardation of one of second furrows. Viewed from animal pole except in *a*; furrows as numbered. *a* and *b*, 10:30 A.M., 5-12-23; *c*, 12 M. Origin of cells in *c* indicated by lettering.

Probably the most common type of irregularity initiated during the second cleavage division is that resulting from retarded or atypical formation of one of the two furrows of the set, so that one reaches the vegetal pole long in advance of the other, as illustrated in Fig. 5, *a*. The animal hemisphere of this egg pictured in Fig. 5, *b* shows that the third cleavage furrows were likewise retarded in the same half of the egg, they being completed in the half on the right while only one had appeared in the opposite hemisphere, assuming that the furrow marked 3 represents a furrow of the third set. In a stage corresponding to that of Fig. 1, *d* the polar cap of micromeres consists of but six cells (Fig. 5, *c*) instead of the eight found in the regular type of cleavage. This egg subsequently developed into a typical fine-celled blastula in which stage it was fixed for sectioning.

In many eggs the cleavage pattern becomes irregular in the course of the third division, after the first two divisions have proceeded in the regular or symmetrical fashion illustrated in Fig. 1. This irregularity may originate through retardation of division in one or more of the blastomeres, as illustrated in Fig. 6, *a*, in which only two micromeres are shown completely separated from the corresponding macromeres. Often, however, the irregu-

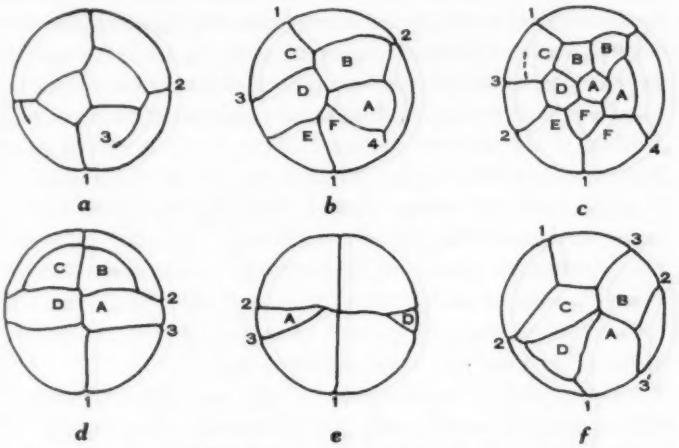


FIG. 6. Eggs showing irregularity of cleavage pattern beginning with third division, after first two have proceeded as in Fig. 1, *b*. Viewed from animal pole except in *e;* cleavage furrows as numbered. *a*, cell with all furrows of the third set horizontal, but furrows delayed in two of the blastomeres. *b* and *c*, egg with two furrows of third set atypical in direction; *b* at 11:45 A.M., *c* at 12:50 P.M. *d* and *e*, egg with two furrows of third set horizontal and the other two parallel to the second furrows in the animal hemisphere (*d*) but meeting the second furrows below the equator (*e*). *f*, egg with furrows marked 3 and 3′ atypical in direction.

larity results from the atypical direction of one or more of the cleavage furrows of the third set. In the egg shown in Fig. 6, *b*, for example, the line marked 3 represents a furrow of the third set which takes a direction parallel with the second furrow, and hence does not cut off a polar micromere in the usual fashion; the third furrow around micromere *A* is also atypical in direction, since it curves back almost to the animal pole instead of joining the first cleavage furrow nearer the equator. In the fourth cleavage

division of this egg micromeres *A* and *B* were divided transversely (see Fig. 6, *c*) and micromeres were cut off from cells *D* and *F*, giving a polar cap of six micromeres, to which a seventh was added later by division of blastomere *C*. The plane of this division is indicated by a dotted line in Fig. 6, *c*. A somewhat more symmetrical pattern was exhibited by the egg shown in Fig. 6, *d*; in this egg two furrows of the third set take the usual position parallel to the equator, while the other two run parallel to the second furrows in the animal hemisphere of the egg but join these furrows in the vegetal hemisphere (Fig. 6, *e*); cells *A* and *D* are thus not typical polar micromeres but extend well toward the vegetal pole of the egg. Still another egg with atypical cleavage furrows of the third set is shown in Fig. 6, *f*. In this egg two furrows of somewhat typical direction had cut off the micromeres *C* and *D*, while the furrow marked 3, meeting the second furrow below the equator, had cut off a much larger cell (*B*). The furrow 3′ ultimately joined the first cleavage furrow some distance below the equator, to cut off another large cell (*A*). This egg thus resembles that of Fig. 6, *d* in possessing only two micromeres of the usual size in the eight-cell stage.

Although only a limited number of eggs were followed through early cleavage, it would seem that irregularity or asymmetry of cleavage pattern in *Hemidactylium* probably begins with the third division in the majority of cases, but with even the first or second in some cases.

Though cleavage in *Hemidactylium* follows the general plan described by Goodale ('11) for *Spelerpes* (*Eurycea*) the vegetal hemisphere of the egg is earlier divided into small cells than in the latter species. According to Goodale the cleavage furrows of the fourth set in *Spelerpes* "do not even reach the lower pole, but usually join the earlier furrows less than 45 degrees below the equator." As a result of this mode of division, the egg of *Spelerpes* in a later stage may show 130 cells visible from above and only 7 from beneath, or 400 above and 14 beneath. In *Hemidactylium* the egg is less heavily yolked than in *Spelerpes* and less markedly telolecithal. The cleavage furrows of the fourth set, though not necessarily reaching the vegetal pole, nevertheless end farther below the equator (see Fig. 1, *e*), while eggs showing 7

or 8 cells when viewed from the vegetal pole show only 25 to 30 when viewed from above. These facts indicate that the egg of *Hemidactylium* is intermediate between that of *Amblystoma* and *Spelerpes,* and far less markedly unequal in its cleavage than the relatively large eggs of *Desmognathus* (Hilton, '09) or *Cryptobranchus* (Smith, '12). In the latter forms the tendency is for the third cleavage planes to be radial or meridional in direction, and the relatively small micromeres of the animal pole arise by later divisions; in *Hemidactylium,* on the other hand, the third cleavage furrows are more frequently equatorial (latitudinal) in direction, and cut off micromeres of relatively larger size, the egg in this respect resembling that of Amblystoma.

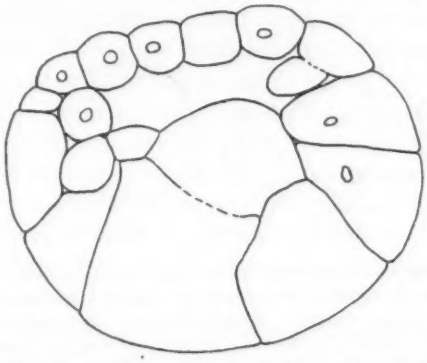


FIG. 7. Early blastula of *Hemidactylium* in vertical section. Outlined with camera lucida. × 31.

So few eggs were available that it was impossible to make a careful study of the internal changes of cleavage. Blastulæ of but two stages were sectioned. The earlier of these, represented in Fig. 7, shows a distinct segmentation cavity roofed by a single layer of cells. It is probable that the blastocoele makes its appearance early, as in the eggs of *Cryptobranchus* (Smith, '26) or *Desmognathus* (Hilton, '09), and as a distinct cavity rather than a collection of intercellular spaces such as Goodale states is frequently the condition in *Spelerpes.* Older blastulæ show a rather characteristic loose arrangement of the internal blastomeres, but a distinct blastocoele is nevertheless present.

Gastrulation and Closure of the Blastopore.

The earliest indication of gastrulation appeared about 90 hours after ovulation in the form of a crescentic depression well down in the vegetal hemisphere (Fig. 8, *a*). This crescent blastopore is bordered by a dorsal lip frequently showing a greater pigmentation than the neighboring surface. The crescent blastopore is gradually extended laterally through further invagination of cells

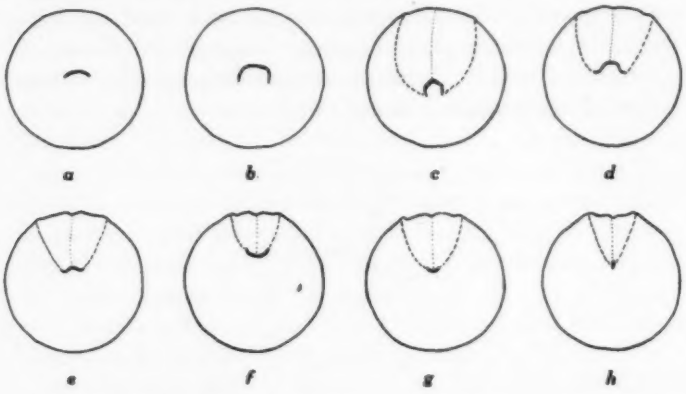


Fig. 8. Diagrams illustrating the formation and "closure" of the blastopore in *Hemidactylium*. No attempt is made to indicate exact relation of blastopore to egg equator. Margin of neural plate indicated by broken lines. *a*, early crescent blastopore; *b*, later crescent blastopore (from another egg); *c*, blastopore of *b* about 24 hours later; *d*, blastopore about 11 hours later than in *c*; *e*, later blastopore from another egg; *f*, blastopore of *e* about 9 hours later; *g*, smaller blastopore from another egg; *h*, blastopore of *g* 10 hours later, reduced to short vertical slit which persists as anus.

at its lateral margins (Fig. 8, *b*) until it assumes the form of a half circumference; sometimes it may become more extensive, as in Fig. 8, *c*, but in no case was the line of invagination or overgrowth observed to become a complete circle surrounding a yolk plug. Following its period of greatest ventral extent the blastopore shortens and assumes the form of a crescent with laterally directed margins (Fig. 8, *d*); by further narrowing and elevation of its lateral portions it assumes the form shown in Fig. 8, *e*, and finally the form of an inverted crescent (Fig. 8, *f*). As the neural folds close the blastopore gradually shortens (Fig. 8, *g*)

and finally assumes the form of a slit elongated in the direction of the body axis (Fig. 8, *h*). It does not appear that the blastoporic opening is lost but rather that it persists as the anal opening.

From the above brief account of the changes in form of the blastopore it may be seen that gastrulation in *Hemidactylium* closely resembles that in *Spelerpes* (Goodale, '11) so far as external features are concerned. Presumably the egg would show the same movements of surface material that Goodale was able to demonstrate in *Spelerpes* by the use of Nile Blue Sulphate; the egg is so lightly pigmented that it, like the egg of *Spelerpes*, might readily be used for studies of this sort. The conclusions of Goodale as to the formation of the germ layers in *Spelerpes* are probably likewise applicable to *Hemidactylium*.

Hemidactylium and *Spelerpes* are possibly unique among American Urodeles in lacking the "yolk-plug stage" characteristic of the development of Amblystoma and the *Anura*. In the larger, yolk-rich eggs of *Necturus* and *Cryptobranchus* the blastopore assumes the form of a complete circle (Smith, '12). Although Hilton claims this to be true also for *Desmognathus*, his Fig. 30 shows a late blastopore of the inverted crescentic form characteristic of the same stage in *Spelerpes* (Goodale, Pl. 1) or *Hemidactylium* (Fig. 8, *f* and *g*). Unfortunately observations on this stage of development of the eggs of *Gyrinophilus* and the Plethodons are not available; possibly in these species gastrulation would follow the pattern found in *Hemidactylium* and *Spelerpes*.

In his account of the development of *Hemidactylium*, Bishop ('18) estimates that his youngest specimen, an embryo showing well-elevated neural folds, is not more than 72 hours old. This estimate would appear to be much too low, assuming that the eggs timed by the writer had not been retarded by the conditions to which they were subjected. In these eggs the early crescent blastopore was first observed about 90 hours after ovulation, and the neural folds did not close until from 60 to 80 hours following this stage, or at from 150 to 175 hours after ovulation. Since temperature determines to a great extent the rate of the developmental processes, and since the laboratory temperature averaged higher than that of the normal environment, it is probable that the eggs observed developed at least as rapidly as would eggs in the field.

For stages of development later than those of Fig. 8 the reader is referred to the excellent sketches published by Bishop ('18). Transections of older embryos of this species are figured in a previous report dealing with the primordial germ cells (Humphrey, '25).

Summary and Conclusions.

1. The egg complement of the *Hemidactylium* female probably requires a period of several hours for its deposition, since the expulsion of a single egg occupies several minutes.

2. The observed position of the body of the female during ovulation (ventral surface upmost) may be of value in preventing scattering of the eggs, since they are thus supported upon the body of the female as well as by adherence to neighboring sphagnum or other eggs.

3. Under laboratory conditions cleavage begins in from ten to fifteen hours after ovulation; it is of the unequal holoblastic type. The first and second cleavage furrows tend to be meridional in direction. The furrows of the third set are frequently horizontal (latitudinal) and lie well above the egg equator, but in many cases they take a direction parallel with the first or second furrows in the animal hemisphere and meet these furrows at some point below the equator. A well developed blastocoele is very early present.

4. Irregularity in cleavage is to be expected in all eggs after the third division. Only occasionally do the eggs show symmerical eight-cell stages, due to the fact that irregularity or asymmetry in cleavage may begin with any of the first three divisions.

5. The vegetal hemisphere of the egg undergoes more division than in the eggs of *Spelerpes, Desmognathus,* or *Cryptobranchus.* In this respect it resembles the egg of *Amblystoma.*

6. Gastrulation begins about 90 hours after ovulation with the formation of the usual crescent blastopore. No ventral lip develops and no yolk plug is formed. The crescent blastopore shortens and becomes inverted, the horns of the crescent pointing upward, and finally closes to a vertical slit which becomes the anus.

7. Closure of the neural folds occurs in from 150 to 175 hours after ovulation. The further course of development has been described by Bishop ('18).

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FERTILE TERMITE SOLDIERS.

HAROLD HEATH,

HOPKINS MARINE STATION,
PACIFIC GROVE, CALIFORNIA.

In two earlier studies (Heath, '03, '27) attention was called to the fact that in the termite genus *Termopsis*, represented in California by two species (*T. nevadensis* and *T. angusticollis*), occasional individuals occur which externally resemble a soldier and yet are sexually mature. In the typical soldier the head is distinctly longer than broad, whereas in the fertile type the two diameters are the same or nearly so. Furthermore the jaws of both project far beyond the labrum, and are built upon essentially the same plan. Hence, unless it is decided that they belong to a special caste, these unusual insects must be looked upon merely as soldiers with fully developed sex organs.

Up to the time when the manuscript of the later paper went to press only four of this type of termite had been discovered. Since then fourteen additional individuals have been collected, and it now appears that they may prove to be of rather frequent occurence. All of these later acquisitions were living in essentially the same conditions. A limb of a Monterey pine (*Pinus radiata*), ten or fifteen feet in length, and evidently occupied by a colony of *T. nevadensis* throughout its entire extent, had broken off near its base, and in falling had broken into several fragments. When the weathering of these, and the depth they had sunk into the leaf mold indicated a sojourn of several months each fragment was carefully opened. A large number of such colonies were examined, and while in most instances complemental royal forms were present, those with soldier-like appearance existed in approximately twelve per cent. of the communities.

In addition to their relatively small heads the behavior of these fertile soldiers is unmistakable. Unlike the typical soldier, which moves about from place to place in the observation nest, the fertile type remains in close association with the normal complemental in-

sects. Also the females are readily distinguished by their distended abdomens. Conjugation was witnessed on six occasions where the soldier paired with an individual of the usual neoteinic type. No especial attempt was made to observe the actual egg-laying

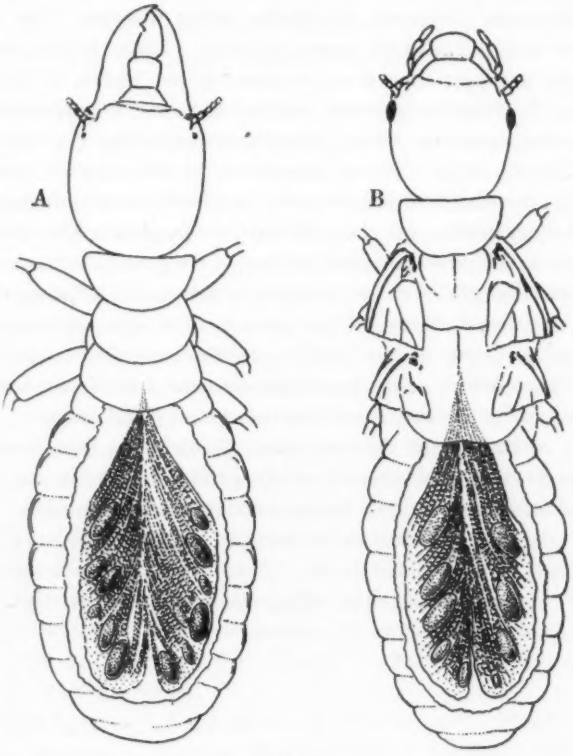

FIG. 1.

process, since this had been observed on two former occasions. Dissections, however, of six specimens showed the sex organs to be well developed. In the text figure, for example, a specimen of female complemental soldier is compared with a primary queen from a colony of *T. nevadensis* two and one half years old. In the first named individual (*A*) there were seven fully-formed ova, including one in the oviduct (not represented), while in the other

specimen the ovary contained eight fully-developed eggs. In addition there were approximately the same number of developing ova in both insects.

In a former account (Heath, '27) some observations were recorded which indicate that the complemental royal forms, at least in the genus *Termopsis,* are fertile soldier nymphs. The fertile soldier also points to the same conclusion. Under normal circumstances it appears that in an orphaned colony insects of this type appear as a rule to be in the penultimate instar, and yet there are occasional specimens which give evidence of belonging to the antepenultimate stage. Also it appears to be true that in some instances the sex gland stimulus may be applied to individuals which have progressed so far along the path to complete soldier development that with the functional activity of the gonad there is a slight suppression only of those characters which normally belong to the fully-developed soldier. This seems to be a reasonable explanation of the origin of these individuals, which are thus seen to combine the activities of the usual complemental royal insect together with many of the structural features of the typical soldier.

Up to the present time no essential differences have been discovered between the offspring of the primary royal pair and those of the complemental royal forms including the fertile soldier. The data relating to this last named category, however, are based upon the study of two colonies only. A report upon a more extensive series of experiments, now being conducted, will be made at a later date.

REACTIONS OF THE CYPRID LARVÆ OF BARNACLES AT THE TIME OF ATTACHMENT.[1]

J. PAUL VISSCHER,

WESTERN RESERVE UNIVERSITY.

Knowledge of the actual process of attachment and of the reaction of the organism immediately preceding this act is very meager for many of the sessile marine organisms. As a large number of these forms cause fouling on ship's bottoms, a careful study of some has been made by the author as part of a study to determine the nature and extent of fouling on ship's bottoms.

The activities of the tadpole larvæ of several species of tunicates have been carefully studied by Caswell Grave (1921–1923). He found that after a free-swimming period which varied from a few minutes to several hours, the larvæ invariably became negative to light and attached by means of adhesive papillæ. The factors which determined when the organisms attached appeared to be related to the physiological state of the organism, while the place of attachment is probably a matter of chance, although the reactions to shadows may determine this in some degree.

Nelson (1924) found "that in the oyster the larvæ 'test out,' by means of their feet, an appreciable area of solid surface before actually attaching, which is preceded by circling movements." It had long been supposed that these larvæ had simply settled and the lucky ones survived.

The only record of attachment of larval barnacles of which the author is aware is found in Darwin (1851). He states that "the larva fixes itself with its sternal surface parallel and close to the surface of attachment, and the antennæ become cemented to it; if the cirripede, after its metamorphosis had remained in this position, the cirri could not have been exserted, or only against the surface of attachment; but there is a special provision that the young cirripede shall immediately assume its proper position at

[1] Published by permission of the U. S. Commissioner of Fisheries. Experiments were performed at the U. S. Biological Station at Beaufort, N. C.

right angles to the position which it held whilst within the larva, namely with its posterior end upwards. This is effected in a singular manner by the exuviation of the great compound eyes, which we have seen are fastened to the outer arms of the double UU-like, sternal apodemes; these together with the eyes stretch transversely across, and internally far up into the body of the larva; and, as the whole has to be rejected or moulted, the membrane of the peduncle of the young cirripede has necessarily to be formed with a wide and deep inward fold, extending transversely across it; this when stretched open, after the exuviation of the larval carapace and apodemes, necessarily cause the sternal side of the peduncle to be longer than the dorsal, and, as a consequence, gives to the young cirripede its normal position, at right angles to that of the larva when first attached."

Barnacles attach to a great many materials and under very diverse environmental conditions. Darwin (1853) stated that " sessile cirripedes adhere to all sorts of objects, floating and fixed, animal and vegetable, living and dead, organic and inorganic," and that " living mollusca are the most frequent objects of attachment." The following list of materials will serve to give some idea of the varied array of materials on which the writer has seen barnacles attached: wood, glass, rubber, beeswax, shells of more than thirty varieties, rocks of many sorts, rope, cloth, leather, metals of many kinds including iron, copper, zinc, lead, tin, aluminum, and to many alloys as brass and bronze, turtles, crabs, lobsters, fucus fronds, bamboo, zostera, sponges, corals, echinoderms, bryozoa, tunicates, and several types of cetacea. It is thus apparent that barnacles attach to almost every type of material submerged in sea-water, which is sufficiently large and permanent to afford a place of growth.

But although barnacles attach to all these materials, there is nevertheless a high degree of specialization among them. Thus *Balanus galeatus* is found only on certain gorgonian corals; *Coronula diadema* only on the humpback whales (*Megaptera*); *Cryptolepas rachianecti* only on the Californian grey whale (*Rhachianectis glaucus*). *Octolasmis mulleri* is found in the gill chambers of several crabs, while *Octolasmis geryonphila* is found only in the gill chamber of a single type of crab (*Geryon quin-*

quedens). *Chelonibia patula* grows only on the carapace of certain crabs (and on *Limulus*) while *Chelonibia testubinaria* grows only on the carapaces of turtles.

Although many thus show a high degree of specialization as regards their habitat, yet within a single genus one often finds wide extremes. *Balanus amphitrite* is perhaps the most widely distributed barnacle existing today, with a world wide distribution, being found in all tropical and warm temperate waters. It attaches to wood, to rocks, to fucus fronds, to rubber, to metals of many sorts and to shells as well as living molluscs of many kinds. In contrast to this, *Balanus galeatus* attaches only to a limited number of gorgonian corals and is typically a West Indian species, although a few specimens have recently been found from Southern California.

The larvæ of all barnacles are hatched as nauplei, the eggs being carried within the mantle cavity of the adult until hatched. These nauplei moult from one to three times during the course of the first week or ten days, changing slightly with each moult into forms known as metanauplei and then metamorphose into an entirely different type of organism called the cypris larvæ or cyprid. The cyprids of all barnacles are bilaterally symmetrical organisms about four times as long as high and often ten times as long as broad. These proportions vary depending upon the genus of the barnacles but the species of any given genus seem to vary but little in this regard.

This free-swimming period as cyprids appears to vary greatly with different forms of barnacles. *Balanus amphitrite* has been observed to attach within seven days after hatching, while *Chthamalus fragilis* probably attaches within four days. *Balanus balanoides*, on the other hand, has a much more extended larval life and apparently does not attach for from 10 to 12 weeks after liberation.

These cyprid larvæ propel themselves through water by means of sudden backward movements of their appendages. These movements occur with great rapidity so that it is quite impossible to see this reaction unless the organisms are swimming very slowly.

They are aided during their pelagic life by the fact that they possess great globules of a fatty substance in the anterior portion

of their bodies. That this is clearly a lipoid in its character has been demonstrated by its reaction with Congo red and with osmic acid. During much of this period this mass of liquid substance seems to act as a buoy holding the larvæ near the surface film. But as the free swimming period draws to a close this substance disappears. It seems probable accordingly that the length of the free-swimming period is dependent upon the amount of this substance originally stored and its rate of disappearance.

Throughout this period of pelagic life the cyprids often swim on their sides, that is, with their oral surfaces on a horizontal plane with their dorsal surfaces. This holds good especially if the organisms are in the surface film at the surface of the water or if they rest on some object at the bottom. They will frequently propel themselves for some time through a considerable distance while in this position. It has also been observed that they usually are found when in this position to have their morphologically right side turned downward while their left side is uppermost.

The actual process of attachment occurs, as suggested above, after a free-swimming period of from three days to two weeks (longer for some species). The cyprids of *Balanus improvisus* and *Balanus amphitrite* have been kept under observation for ten or eleven days at the end of which time some were found to have attached but many were still active.

When the internal physiological conditions necessary for attachment are present, conditions apparently correlated with the "lipoid" content of the organisms, the larvæ have been observed on many occasions to "walk" on the substratum apparently "hunting" a place for attachment. This remarkable performance is accomplished by alternate attachment and release of the adhesive tips of the antennæ combined with a "push" from the set of appendages.

By reference to Fig. 1, it will be seen that when one of the antennæ (a–2) is released the appendages (ap) straighten out, throwing the larva forward. The released antenna (a–2) then attaches, while the appendages are withdrawn, and with the next move the antenna (a–1) is released.

In this manner these organisms have been observed to traverse a distance of more than twelve mm. (about ½ inch) and have been

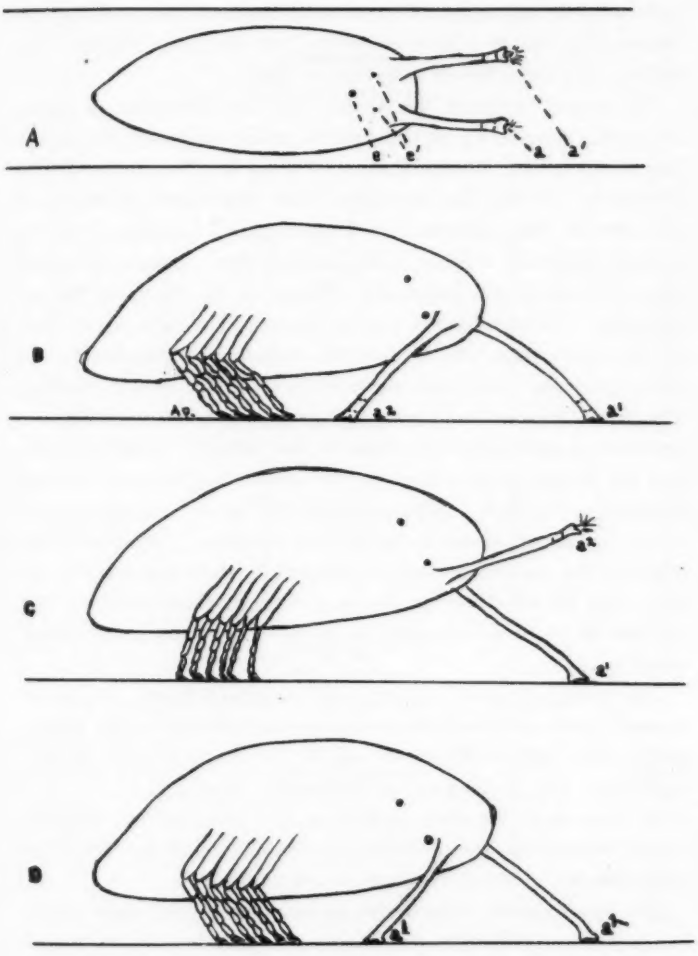

FIG. 1. To illustrate the movements of barnacle larvæ at the time of attaching. *A:* ventral view to show antennæ, a' and a^2; median eye, *e*, and the paired lateral eyes, e'. *B–D*, lateral views of cyprid larva, showing method of locomotion at time of "selecting" a place for attachment.

seen to "test" different areas for a period of more than one hour before finally attaching. Frequently it has been observed that the antennæ appear to adhere so firmly that the larva "kicks" for eight or ten times before releasing its hold.

On several occasions the writer has been fortunate in seeing the actual process of metamorphosis while observing through a microscope giving a magnification of about one hundred and fifty diameters. It was then seen that after attachment by means of the antennæ, the organisms "kick vigorously" for some time, but without effecting release. The animal then appears to settle down as soon as attachment was effected, in the region of the appendages. Metamorphosis now occurred. The two-valved shell of the cyprid stage was thrown off, including the exoskeleton of the appendages (often also the paired eyes). From the resulting almost amorphous mass the young barnacle is soon made out. A secretion is continually laid down on the formerly ventral surface, and the rudiments of a coating (the future shell) appear around the sides of the mass. After one hour the "head" and appendages of the animal can be seen in an inverted condition. Whereas when attached the appendages extend downward, they now extend upward and the mouth parts too have changed their position; this reversal in position occurring as described by Darwin as quoted above.

No opercular valves appear until after six hours, while the separate plates of the shell do not appear until more than twelve hours after metamorphosis, in any of the observed cases of *Bal. amphitrite, Bal. improvisus,* or *Chthamalus fragilis.*

In these types the plates appear as four units taking the place of the original apparently chitinous material which protected the organism during the early stages of metamorphosis.

The cyprid larvæ of barnacles are also sensitive to light and it has been shown (Visscher and Luce, 1928) that they are stimulated to a much greater degree by a given amount of energy in the field of green than by like amounts of energy in other fields of the spectrum. These larvæ are usually positive in their reactions but in the later stages they are very erratic and may not show either positive or negative reactions for a considerable period of time. However, at the time of attachment, although still sensitive to

light and especially to wave-lengths of 470–500 $\mu\mu$ they become decidedly negative and move away from the source of stimulation.

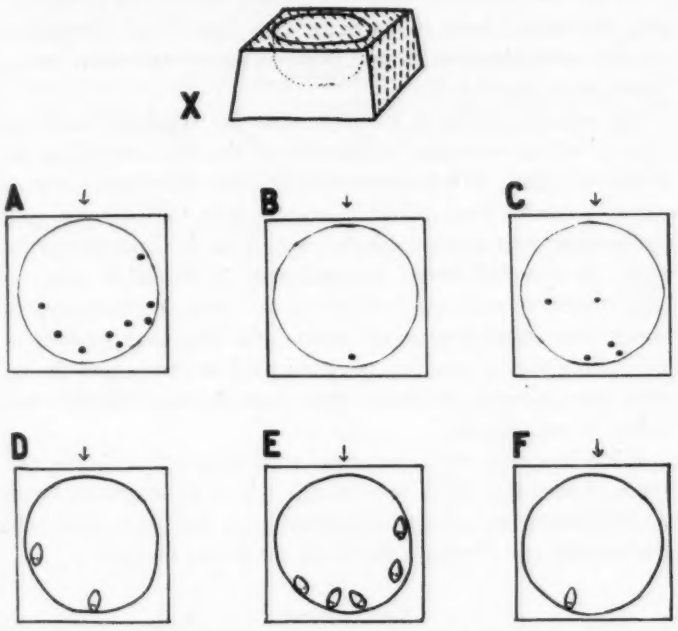

FIG. 2. To illustrate effect of light at time of attachment of larval barnacles. *X*, glass dish, 4 cm. square, by 1.5 cm. in height, with concave depression 2½ cm. in diameter and 1 cm. in depth, covered with heavy black paper on five of its six sides as indicated. *A–C*, and *D–F*, two series of tests, contained barnacle larvæ, in which each container was covered as in *X*, and with the exposed side toward the window as indicated by the arrow. Only those larvæ which had attached and metamorphosed are indicated.

It has been impossible to select cyprid larvæ of known age, but the following experiment will demonstrate this reaction. A number of cyprid larvæ were isolated from a collection of tow made in the tidal channel in front of the laboratory island. From the original lot those with the least amount of "oil droplets" were selected and placed in small glass dishes resembling salt cellars which were surrounded on five of the six sides with a dull black paper (Fig. 2). These were then set on a table in front of a large

north window with the uncovered side of the dish toward the window.

In the first series of three experiments twelve cyprid larvæ of *Bal. improvisus* were placed in *A*, three cyprids of *Chthamalus fragilis* were placed in *B*, and between twenty and thirty mixed forms were placed in *C*.

By referring to Fig. 2, it will be seen that attachment occurred almost without exception on the side of the dish away from the source of light. It was observed at this time that many of these young barnacles were definitely oriented with their anterior ends (containing their eyes) decidedly away from the light side of the dish. In a second series of experiments *D*, *E*, and *F* (Fig. 2) this feature is even more clearly seen. Although these experiments were repeated some two weeks later with large numbers of individuals only a very few were observed to attach, and the results were no more conclusive than those shown in the first two series of experiments.

It can be clearly seen from these experiments that for the two types of barnacles which were tested, light is an important factor in determining the point of attachment, and that they orient with the anterior end directed away from the source of light.

Conclusions.

Barnacle larvæ are sensitive to light at the time of attachment and for the three species tested, are negative to light at this time. It has also been demonstrated that barnacle larvæ do not attach at random, *i.e.*, merely by chance, but that they apparently "test out" the surface before attaching, in which process they have been observed to spend a very considerable period. The antennæ are evidently the "feelers" as well as the final "hold-fasts" and once definitely attached a secretion is deposited which protects the young barnacle from the beginning of its life as a sessile organism.

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REACTIONS OF THE CYPRID LARVÆ OF BARNACLES TO LIGHT WITH SPECIAL REFERENCE TO SPECTRAL COLORS.[1]

J. PAUL VISSCHER AND ROBERT H. LUCE.

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INTRODUCTION.

It is well known that the larvæ of barnacles as well as the larvæ of almost all sessile marine organisms are, at times, sensitive to light and are either photopositive or photonegative. This fact has led to their use for experimental purposes, but the use of the larval forms of barnacles in such experiments has been confined to the earliest free swimming stage call the *Nauplius*. No records have been found of the use of the later larval stage, the cyprid larva in experiments with light.

Since barnacles are the most important of the various types of organisms which cause fouling (cf. Visscher, 1928) and since barnacles attach while in the cyprid stage and subsequently metamorphose into the adult form and since it had been observed on ship's bottoms that barnacles were frequently most numerous on the shaded area of the hull, it seemed important to ascertain the stimulating efficiency of light of different wave-lengths in regard to the cyprid larvæ of barnacles.

In previous work on the reactions of various organisms to color, much confusion has arisen from the fact that the apparatus used was neither standardized nor calibrated. Where spectral transmissions were known, there was frequently no attempt to balance

[1] Published by permission of the U. S. Commissioner of Fisheries. Experiments were performed at the U. S. Biological Station at Beaufort, N. C.

the radiant energies. The only occurrences of the use of different spectral colors with balanced radiant energies are those of Pergens (1899), Blauuw (1908), Laurens (1911), Day (1911), and Gross (1913).

The most common error in the earlier work was that of ascribing the effect of colored light to its wave-length, and ignoring the effect of intensity. Pergens was the first to recognize the difference between wave-length and intensity. Working with a spectrometer he found that the energy of the blue end of the spectrum was too small to be measured. He then used light filters of colored glass, and measured the light intensity with a Ritchie photometer. Pergens failed, however, to eliminate the effect of the infra red transmitted by the filters.

Laurens, Day and Gross used apparatus which was essentially alike. The light was diffracted and balanced and the band of transmission was either narrowed or widened until the deflection of the galvanometer was the same for all the colors. While this work was done with colors of high purity and balanced radiant energies, it cannot give more than a relative idea as to the stimulating efficiency of parts of the spectrum, as only four limited regions were employed.

Mast (1917) used a Hilger spectrometer and a Lummer-Brodhun rotating sector and was able to determine with great accuracy the relative stimulating efficiency of the various parts of the spectrum. While this method allows a direct study of the effectiveness of spectral colors, it appeared to be limited in its application to organisms which, in a single beam of light travel parallel to the rays and do not deflect to either side and consequently could not be used with cyprid larvæ. The intensity of light obtainable by this method was believed to be too limited for adequate work on the cyprid larvæ of barnacles. For these reasons and others, to be discussed later, it was considered inadvisable to use this method in the following work.

Materials and Methods.

The larval life of the barnacle is divided, with a very few exceptions, into two distinct stages, the nauplius, and the cyprid. The cyprid stage is characterized by the presence of a large bi-

valve shell, giving it a general resemblance to certain ostracods. This resemblance, however, is superficial and does not extend to the limb structure or internal anatomy. All of the appendages of the adult are present; the antennules protrude from between the valves of the carapace; there are six pairs of thoracic limbs, but no abdominal appendages. The unpaired eye of the nauplius is usually still present, and in addition there is a pair of large compound eyes.

Cyprids of various species of barnacles differ from each other in the size and proportions of the carapace, and in the presence of certain characteristic bodies within the carapace. During the time of year in which this work was done, three main types of cyprids predominated. These types were of two distinct sizes. The smaller size was .51 mm. long, .225 mm. high, and .20 mm. wide, and was found by subsequent metamorphosis to be the cyprid of *Chthamalus fragilis*. The larger type was made up of two groups which were outwardly very similar, being .65 mm. long, .24 mm. high, and .20 mm. wide. These groups could only be distinguished by the presence of certain granules in the carapace. One of these was determined by subsequent metamorphosis to be *Balanus improvisus*, and the second was the cyprid of *B. amphitrite*.

Balanus improvisus and *B. amphitrite* are known to breed most abundantly at Beaufort during June and July, and large numbers of their nauplii and cyprids are to be found at that time. *Chthamalus fragilis* has been observed to have a more extended breeding period, apparently running from May into late September. *Balanus eburneus* is abundant at Beaufort, but as far as is known, no cyprids of this species were used in the experiments.

Cyprids were obtained by towing with a number 12 mesh net. They were picked out of the tow by means of a capillary pipette, then washed in clear sea water and placed in the aquarium. During the summer of 1924 cyprids were not numerous but during the summer of 1925 cyprids were very abundant, comprising at times in late June and early July a large part of the plankton. This abundance allowed a selection of material, and in consequence most of the work was carried out with the cyprids of *B. improvisus* and *B. amphitrite*. Cyprids appear to be most abundant

on an incoming tide and at flood tide. This is also true for the nauplii during June when they were most abundant. There is a marked diurnal migration, so that tows during the day must be made with the net about eight feet below the surface, this being slightly less than the minimum depth of the channel. The time of exposure to sunlight exerts a marked influence on the activity of those cyprids which remain at the surface. Cyprids taken from a surface tow during the morning are fairly active, but those taken from a surface tow during the late afternoon are inactive, and could not be used.

The apparatus used in the following experiments consisted of 100 watt, gas-filled, tungsten lamps, two copper sulphate cells, and thirteen monochromatic light filters.

In selecting the filters, an attempt was made to use only those having a narrow transmission band. This is somewhat difficult to obtain for the yellow and blue-violet. Wratten filters were available for most of the needed colors, but these filters are not wholly stable under the climatic conditions found at Beaufort. At the suggestion of Dr. K. S. Gibson of the Bureau of Standards, we were able to obtain a number of filters from glasses manufactured by the Corning Glass Works, which company very kindly supplied them for our use. These glasses were ground to thicknesses giving transmissions in accordance with the curves shown in Bureau of Standard Technologic Paper 148. The glasses are more satisfactory than Wratten filters because of their permanency under all climatic conditions. However, Corning glasses were not available for certain regions of the spectrum, so four Wratten filters were used, making a series of thirteen filters covering the whole visible spectrum. By referring to figure No. 1 a list of all "Corning" filters with their curves of transmission can be found. Table I. is a summarization of the curves for all filters used giving the limits of transmission and the dominant wave-length of each filter.

The difficulty encountered in obtaining a deep blue or ultraviolet arises from the fact that all the filters in this region transmit some of the red end of the spectrum. No filter was available to block out the red without also cutting off some of the blue or ultraviolet. Since red is relatively feeble in stimulating effect, it

was judged best to use the blue and untraviolet filters as they were, and to attempt a correction for the presence of the red later. We were not able to obtain a yellow filter with a sharply limited transmission band, since ordinary yellow filters merely cut off a portion of the blue end of the spectrum. Perhaps the best yellow would be produced by a combination of Corning glasses G36

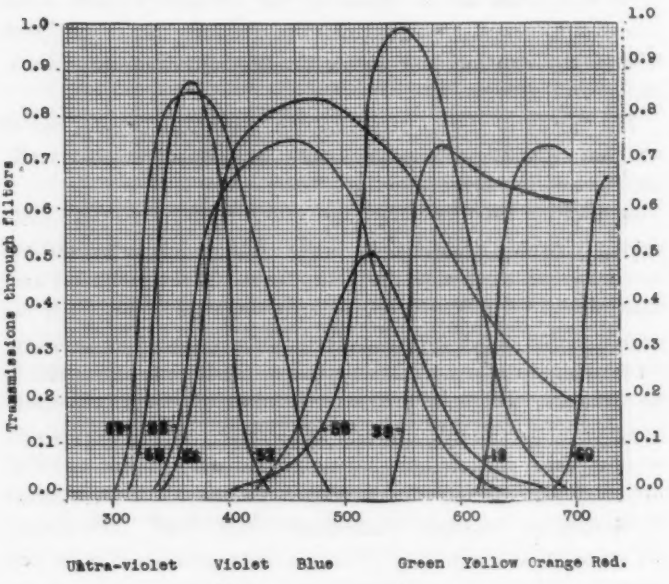

FIG. 1. Curves to illustrate the range of light energy transmitted by the "Corning" glass filters used to test the relative stimulating efficiency of spectral colors. (From the U. S. Bureau of Standards Technologic Papers No. 148.)

(curve 38) and 401Z (curve 59) as given in Bureau Standards Paper No. 148. Unfortunately, however, this combination was not available to us at the time. The balancing of the radiant energies for all filters was done by the Bureau of Standards, by the use of an apparatus based upon the radiomicrometer of Boys.[1]

[1] The authors are grateful to the Director of the Bureau of Standards and also to Dr. W. W. Coblentz for their kindness in calibrating all filters and lamps used for these experiments.

TABLE I.

LIST OF FILTERS USED IN EXPERIMENTS, SHOWING THEIR TOTAL SPECTRAL TRANSMISSION, AND THEIR DOMINANT WAVE-LENGTHS.

Filter.	Total Transmission.	Dominant Wave Length.
Ultra C 83	315–428 μμ 609–Red End	355 μμ
Purple C 69	310–485 μμ 690–Red End	370 μμ
Purple W 35	300–475 μμ 650–700μμ	420 μμ
Blue W 49	400–510 μμ	440 μμ
Blue C 60	335–640 μμ	460 μμ
Blue C 59	335–690 μμ	480 μμ
Blue Green C 56	340–700 μμ	505 μμ
Green C 52	425–670 μμ	530 μμ
Green W 58	485–635 μμ	540 μμ
Yellow W 15	500–700 μμ	590 μμ
Orange W 22	545–700 μμ	620 μμ
Orange C 38	540–Red End	640 μμ
Red C 19	620–Red End	700 μμ

The letter "C" after a filter denotes a Corning glass filter. The numbers after the Corning glasses refer to the transmission curves shown in Bureau of Standards Technologic Paper 148. The letter "W" denotes a Wratten filter, and the number refers to the transmission curves found in the booklet "Wratten Filters" published by the Eastman Kodak Company.

Each lamp to be calibrated was set up with the center of the curved filament facing a radiometer. The radiometer was placed on a scale graduated in centimeters, and could be moved toward or away from the light. Connected with the radiometer was a Coblentz modification of Thompson's galvanometer. This was hung on the wall, fifteen feet from the radiometer, and at right angles to it. The lamps used were 100 watt, 115 volt, gas-filled, Mazda lamps and during the test each lamp carried 109.3 volts and 0.87 amperes. In these experiments the infra-red was absorbed by copper sulphate cells. Each cell was two centimeters thick, and consisted of two pieces of optical glass cemented to a glass ring, five centimeters in diameter. The cells were filled with a solution consisting of 57 grams $CuSO_4$ in two liters of water. One of these cells was placed in front of each lamp tested. The radiometer was then moved back and forth until the galvanometer showed a deflection of one centimeter. This was taken as a

standard deflection. A filter was then placed before the lamp and the galvanometer again brought to a deflection of one cm. Such balancing of the radiant energies, therefore, eliminates the necessity of correcting for the unequal distribution of energy in the spectrum of the lamp. The result of any experiment conducted with these balanced filters represents, therefore, the actual stimulating efficiency of the specific wave-length of the light, and is not in any way influenced by a difference in light intensities.

The apparatus was set up so that two beams of light crossed at right angles (Fig. 2). The lamps were enclosed in light-proof boxes each having an opening five centimeters square. A copper sulphate cell was placed in front of each of these openings. Each box was readily moved, and the center of the crossing of the two beams was used as the zero point from which to measure the position of the light. The aquarium in which the organisms were placed was made of the best quality slide-glass, and was 35 mm. square and 10 mm. deep. This aquarium was placed on a stand at the center of the two cross beams. Underneath this aquarium was placed a small microscope lamp which could be lighted momentarily so that the cyprids could be counted more readily. Two screens were placed just outside of the aquarium stand, one of these had an opening admitting white light, and the other had a slot in which the filters were placed. In practice, the lamp giving the white beam was placed permanently with the center of the filament at a point 119.2 cm. distant from the center of the two beams of light while the lamp used with the filters was moved to such distances as to compensate for the energy transmission value as previously determined for each filter.

Ten cyprids were placed in each aquarium in carrying out an experiment. They were allowed to become dark adapted, and were then given a one minute exposure to light. At the end of this time the cyprids in the white light, in the filter light, and in the corner between the two light beams, were counted. These numbers thus obtained were recorded in a table of four columns under the heads of White, Corner, Filter, and Non-reacting. A dark period of about twenty seconds was given between trials. Three trials were made with each filter, after which the filters

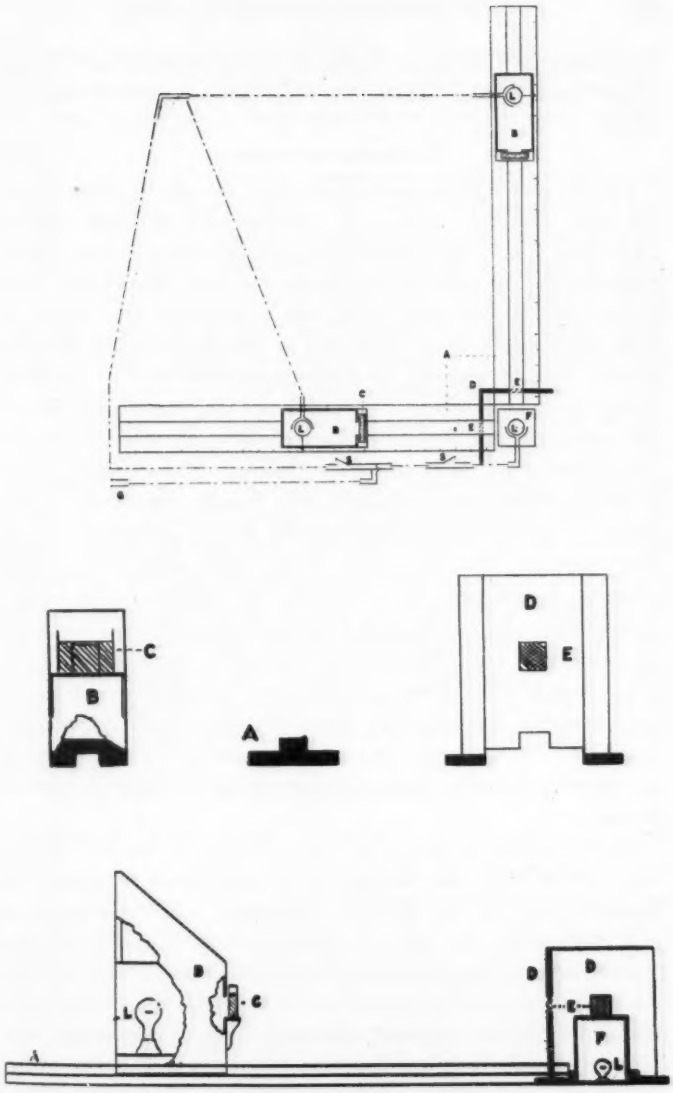

FIG. 2. To illustrate apparatus used to test the relative stimulating efficiency of equal amounts of light energy, in known and limited fields of the spectrum, upon the cyprid larvæ of barnacles. *A*, Track for boxes (*B*) containing the electric bulbs (*L*). *C*, copper sulphate filters in front of square opening in light box (*B*). *D*, screen holding filters (*E*) and surrounding aquarium stand (*F*). *S*, switches for control of lights. *G*, feed line for electric current.

were changed. The same cyprids as a rule could be used for the whole series of filters, though certain factors modified their behavior.

Behavior of Cyprids.

Cyprids give definite reactions to light for two to three hours, but after that the number of non-reactants increases rapidly. They must also be dark adapted in order to secure definite movements toward the light, but apparently there are limits to this dark adaptation. It was noted during the experiments that if the cyprids were left in total darkness for periods of thirty to sixty minutes, that on resuming the experiments there were no reactants during the first exposure. In the second exposure there were a few reactants, but several exposures were required before the usual number of reactants were obtained. This, however, can be demonstrated more strikingly with nauplii, where, in several instances, ten exposures were required before all of the nauplii were reacting. The effect of a shorter dark period is also well marked. A dark period of one minute was used during the earlier experiments. By varying this rest period, however, and counting the number of reactants and non-reactants, it was found that a rest period of about twenty seconds gave the smallest number of non-reactants. Such a period varies somewhat as the experiment progresses, for a shorter period can be used in the first part of the experiment, and a slightly longer period is necessary toward the end.

Cyprids usually lie upon either side when not in motion. Movement is usually on the side, and is brought about by rapid and powerful kicks of the thoracic appendages. The movement is rapid and usually in a wide arc, but sometimes in a straight line. It is not at all unusual for them to turn about in a complete circle or series of circles before shooting off toward the light. The position of the antennæ apparently has much to do in determining the direction of movement, the movement being in a wide arc or fairly straight line when the antennæ are withdrawn into the carapace, and circular when they are extended. The cyprids may either sink down and lie quietly on the bottom after striking the side of the aquarium, or they may move back and forth along the side of

the dish in a series of jerky movements. This uncertainty in their movements makes it necessary to have a definite time of exposure, and also necessitates a rapid counting of those collected in each light.

The presence of small oil droplets in the anterior end of the cyprids modifies their behavior to a considerable extent. These droplets are usually present, and are found either in the anterior end or along the dorsal side. Their size may apparently be taken as an indication of the age of the cyprid. Cyprids about to attach have very small drops, are sluggish, and move about on the bottom of the dish with a walking movement of the antennæ and a push or slow kick of the thoracic appendages. Those in which the oil droplets are large, often become caught in the surface film. When this occurs it is difficult to get them out, and while they may be very active, they cannot react definitely toward the light. Their reaction consists of spasmodic kicks and short movements toward the light, but these movements soon cease and they drop back passively toward the center of the dish. If two or more cyprids get into the surface film, they cluster together and are of no further value in the experiment. In such cases they were removed and replaced by fresh organisms. The cyprids which are the most favorable for study are those which are too old to get in the surface film, and which are not ready for attachment.

Results.

In Table II. are shown the results of twenty series of experiments as described above. These results are summarized in figure 3 showing a curve which clearly indicates that there is a fairly wide region in the spectrum from 505 to 590 $\mu\mu$ or from light blue to yellow, where the stimulating efficiency is equal to, or more than 50 per cent. of white light. The maximum of this region is in the light green from 530 to 545 $\mu\mu$ where the stimulating efficiency is between 90 and 94 per cent., or practically the equivalent of white light.

In the region of the spectrum from the yellow-orange through the red end, or from 590 to 700 $\mu\mu$, there is a very sharp drop in the effectiveness of light, until at 700 $\mu\mu$ this is less than 5 per cent.

of white light. On the other side of the maximum there is a fall in value from 480 to 440 $\mu\mu$, but this is not as sharp as on the red end. From 420 into the ultra-violet there is a flattening of the curve which indicates strongly that ultra-violet light has a distinct stimulating effect. This effect, however, is very limited in comparison with the maximum in the field of green.

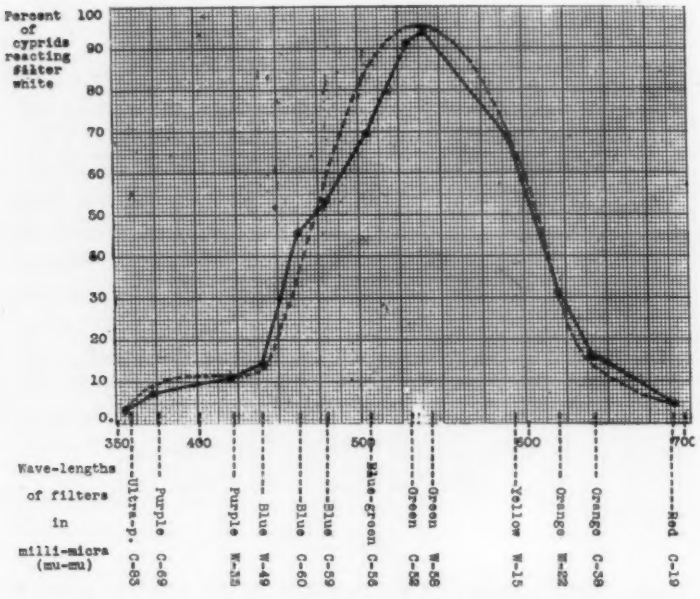

FIG. 3. A curve showing the distribution of the stimulating efficiency of equal energy values among the various parts of the spectrum, for the cyprid larvæ of *Balanus amphitrite* and *Balanus improvisus*.

Due to the fact that light from a tungsten filament does not penetrate far into the ultra-violet, we were unable to test this region as carefully as desired, but in all tests the relative energy values were carefully calculated.

No filter transmitting ultra-violet was available which did not at the same time transmit some light in the far-red end of the spectrum. (Compare No. 69 in Fig. 1.) The efficiency values for these filters were therefore corrected for the presence of red by

TABLE II.

RESULTS OF TWENTY SETS OF EXPERIMENTS TO DETERMINE RELATIVE EFFECTS OF EQUAL LIGHT ENERGIES IN DIFFERENT FIELDS OF THE SPECTRUM, UPON THE CYPRID LARVÆ OF BARNACLES.

Number of Filter	Ultra C 83		Purple C 69		Purple W 35		Blue W 49		Blue C 60		Blue C 59		Blue C 56		Green C 52		Green W 58		Yellow W 15		Orange W 22		Orange C 38		Red C 19	
Dominant Wave Length	355 μμ		370 μμ		420 μμ		440 μμ		460 μμ		480 μμ		505 μμ		530 μμ		540 μμ		590 μμ		620 μμ		640 μμ		690 μμ	
Limits of Transmission	315–428 μμ 690– Red End		310–485 μμ 690– Red End		300–475 μμ 650–700 μμ		400–510 μμ		335–640 μμ		335–690 μμ		340–700 μμ		425–670 μμ		485–635 μμ		500–700 μμ		545–700 μμ		540– Red End		620– Red End	
Number of Trial.	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter
1	27	3	25	3	28	2	30	3	20	7	28	13	19	10	14	13	14	15	13	11	20	12	24	6	31	4
2	28	2	27	5	26	2	25	5	14	10	30	10	19	12	19	16	20	15	24	14	20	9	28	11	34	4
3	33	1	28	1	28	2	30	4	19	7	18	12	16	11	21	19	18	15	19	15	19	7	29	7	34	1
4	30	3	30	3	32	2	24	3	18	10	21	11	19	12	17	16	19	14	23	16	18	8	23	3	33	2
5	26	1	24	2	26	4	23	2	14	9	13	10	14	10	18	17	19	15	21	13	20	7	26	6	32	2
6	29	1	27	2	27	4	24	3	14	6	16	7	15	11	13	10	19	15	23	15	25	6	24	4	27	2
7	22	2	27	1	27	4	28	5	23	9	23	10	22	14	16	22	16	15	22	18	25	6	33	6	35	1
8	36	2	32	3	26	3	32	4	24	9	20	10	13	11	12	15	17	16	20	13	29	5	24	3	26	1
9	20	1	18	2	28	3	22	4	18	6	17	14	15	13	15	16	19	17	19	11	26	5	30	5	33	1
10	32	2	33	4	25	3	29	4	20	14	18	12	16	12	14	15	17	14	19	14	24	6	22	2	32	1
11	32	1	30	1	25	4	32	5	19	10	18	14	21	15	21	19	11	15	17	9	20	7	24	3	25	2
12	35	1	32	3	22	4	33	2	22	9	24	12	21	15	20	15	16	10	20	11	24	7	26	2	30	1
13	29	2	28	2	27	4	28	3	23	8	22	10	21	15	18	15	18	14	17	13	23	8	25	5	30	0
14	30	1	27	0	29	3	32	3	20	14	15	8	20	10	22	17	17	19	15	10	22	6	29	3	29	1
15	36	0	32	2	25	5	32	3	20	8	20	7	18	15	18	18	15	15	22	15	18	6	29	4	37	1
16	34	1	31	2	25	4	30	5	23	13	21	12	24	17	21	17	12	11	20	14	22	7	24	3	29	2
17	34	1	33	1	26	4	28	4	30	5	24	8	26	10	20	18	12	14	19	14	21	4	30	4	31	1
18	35	2	27	4	26	2	27	6	20	11	21	13	19	14	20	17	13	19	21	13	21	8	27	3	31	0
19	25	1	32	2	23	4	31	4	25	13	23	16	25	16	20	17	12	20	14	12	24	8	27	5	30	1
20	30	0	29	2	27	3	26	3	25	10	28	15	18	12	18	15	13	12	19	15	20	9	33	3	35	0
Total number of reactants to either light	603	28	572	51	528	66	534	75	411	188	420	224	381	265	357	327	319	300	387	266	453	141	537	88	622	28
Per cent. of stimulating efficiency as determined by dividing the number reacting to white with the number reacting to filter	4.6% Corrected for Red 3.2%		8.9% Corrected for Red = 7.4%		12.5% Corrected for Red = 10.9%		14.2%		45.8%		53.4%		69.6%		91.6%		94.0%		68.7%		31.1%		16.4%		4.5%	

determining the percentage of red transmitted and then deducting that value from the total value on the basis of the effectiveness of that region in the red.

During the course of this work on cyprids it was also found possible to test the reactions of the nauplii of four species of barnacles to these lights as described above. These results were uniform in that the region of the spectrum between 530 and 545 $\mu\mu$ was in each case the most stimulative. This maximum stimulating effect was the same both when these organisms were negative and when positive to light.

It is of interest that our results are in substantial agreement with the work of other investigators using nauplii of barnacles: Loeb & Maxwell, 1910, Loeb & Westeneys, 1916 and Loeb & Northrup, 1917. Thus it is apparent that the same region of the spectrum offers the greatest stimulating efficiency in both the nauplius and in the cyprid which is not surprising in view of the fact that the nauplius eye is still present in the cyprid.

Conclusions.

The cyprid larvæ of barnacles agree with the larvæ of most other marine organisms in that they react to light. With equal radiant energies and with the infra-red heat rays eliminated, a distinct difference in stimulating effectiveness is found between different regions of the spectrum. This difference shows, for the cyprids of the two species of barnacles tested, a region of maximum efficiency in the light green, between 530 and 545 $\mu\mu$. It is of interest to note that similar results have been found for the larvæ of other marine organism. The larvæ of the hydroid, *Eudendrium* are most sentitive to blue light (460–480 $\mu\mu$); the larvæ of *Arenicola*, to the green (about 495 $\mu\mu$); and the larvæ of the squid, to blue green (470–510 $\mu\mu$).

However from the results obtained by Hess (1910), Mast (1917) and Hecht (1921), it is apparent that the distribution of stimulating efficiency in the spectrum is not necessarily the same for all organisms, which react to light. It is consequently apparent that there is no true color vision in these lower forms—in the usual interpretation of the term, but rather that the photo-sensitive materials in the light perceiving organs are more sensitive to the

effects of certain wave-lengths than to others. Increased intensity of other wave lengths may however produce similar results.

This investigation has, however, an important bearing on the problem of the nature and extent of the fouling of ships' bottoms, an intensive study of which is being completed by the senior author and of which this work forms a part. It has been found that light is an important factor governing the attachment of the organisms which cause fouling and that at the time of attachment many of these larvæ are negative to light, for they are found most abundantly on the shaded portions of the ships' hull.

Since barnacles are the most important factor in fouling it seems probable that the studies reported above may have some significance in the solution of this problem, for if a light green paint were used in place of the red, now universally used, a considerable benefit should be derived, on theoretical grounds, provided all other factors were comparable.

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